SECURITY CLASSIFICATION OF THIS PAGE

AD-A200 034

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION: UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE: 1 0 6 1988	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S): AFOSR-TR- 88-1013
6a. NAME OF PERFORMING ORGANIZATION: University of Georgia	6b. OFFICE SYMBOL (If applicable)
6c. ADDRESS (City, State, and ZIP Code): Athens, GA 30602	7a. NAME OF MONITORING ORGANIZATION: AFOSR-NL
	7b. ADDRESS (City, State, and ZIP Code): Bolling Air Force Base, DC 20332-6448 Bldg 410
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: AFOSR	8b. OFFICE SYMBOL (If applicable): NL
	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: AFOSR 870248
8c. ADDRESS (City, State, and ZIP Code): Bolling Air Force Base, D.C. 20332-6448 Bldg 410	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
61102F	2312	A5	

11. TITLE (Include Security Classification)
Validation and Application of Pharmacokinetic Models for Interspecies Extrapolations in Toxicity Risk Assessments of Volatile Organics

12. PERSONAL AUTHOR(S) Dallas, Cham Edwards; Bruckner, James Victor; Gallo, James; Raghupathy Ramanathan; Srinivasa Muralidhara

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 7/1/87 TO 6/30/88	8/30/88	113

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Physiologically-based pharmacokinetic model, Saturable metabolism, Respiratory elimination, Halocarbon inhalation exposure, Halocarbon oral exposure, Interspecies extrapolations, Pharmacokinetics, 1,1,1-trichloroethane, 1,1-dichloroethylene

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

In pursuit of the goal of establishing a scientific basis for the interspecies extrapolation of pharmacokinetic data in health risk assessments, a series of studies have been conducted involving pharmacokinetic determinations in rats (to be followed later in dogs) to several aliphatic halocarbons. Direct measurements of the uptake and elimination of halocarbon in the blood and exhaled breath of rats have been completed during and following inhalation exposures to trichloroethylene (TCE), trichloroethane (TRI), and dichloroethylene (DCE). Two manuscripts have been completed for publication in peer-reviewed journals on the work with inhaled TCE and TRI. Pharmacokinetic determinations have also been made in studies of the ingestion of TCE, TRI, and DCE. A physiologically-based pharmacokinetic (PBPK) model has been developed for the computer simulation/prediction of the pharmacokinetics of halocarbons in rats. Overall, values predicted by the PBPK model for halocarbon levels in the blood and exhaled breath for inhalation exposures and for blood levels following ingestion were in close agreement with measured values in the rat in the current studies.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Major T. Jan Cerveny	(202) 767-5021	AFOSR-NL

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

08 10 5 292

01 SEP 1988

Block 18 - SUBJECT TERMS (Continued)
trichloroethylene, methyl chloroform, volatile organics

VALIDATION AND APPLICATION OF PHARMACOKINETIC MODELS FOR INTERSPECIES EXTRAPOLATIONS IN TOXICITY RISK ASSESSMENTS OF VOLATILE ORGANICS

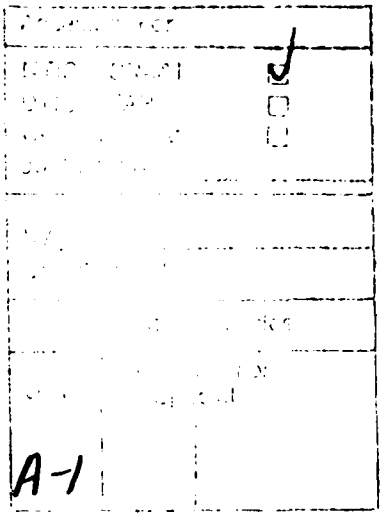
Cham E. Dallas, Ph.D.
Department of Pharmacology and Toxicology
College of Pharmacy
University of Georgia
Athens, GA 30602

August 30, 1988

Annual Report for Period 1 July 1987 - 30 June 1988

Prepared for

Life Sciences Directorate
Air Force Office of Scientific Research
Bolling Air Force Base, DC 20332-6448



I. OVERALL OBJECTIVE AND STATEMENT OF WORK

The overall objective of the proposed project is to investigate the scientific basis for interspecies extrapolation of pharmacokinetic and neurobehavioral toxicity data. Direct measurements of blood and tissue concentrations of halocarbons over time in two species will be used to formulate and validate physiologically-based pharmacokinetic models for inhalation and oral exposure. These models will be used for: (a) prediction of the time-course of blood and target organ levels in the absence of data; (b) interspecies extrapolations (i.e. scale-up from smaller to larger laboratory animals and ultimately to man). A combined physiological pharmacokinetic-toxicodynamic model for inhalation exposure to halocarbons will also be developed and evaluated for its ability to predict neurobehavioral effects under specified exposure conditions.

A series of experiments will be conducted to provide a pharmacokinetic data base of interspecies comparisons and for formulation of physiologically-based pharmacokinetic models. Adult male Sprague-Dawley rats and male beagle dogs will be administered equal doses/concentrations of selected halocarbons. Ingestion, inhalation and intravenous injection will be employed as routes of administration. concentrations of the parent compounds will be monitored in the blood (and in some cases in the exhaled breath) for appropriate periods during and after exposures. The cumulative uptake from exposure to each chemical will be determined. Relative rates and magnitude of elimination of the test chemicals by metabolism and respiration will be evaluated. For investigating the relative role of metabolism on the observed pharmacokinetics of volatile organic compounds, trichloroethylene (TCE), dichloroethylene (DCE), and trichloroethane (TRI) will be employed. Tetrachloroethylene (PER) and 1,1,2,2-tetrachloroethane (TET) will be employed for analyzing the role of pulmonary extraction/elimination. At least 2 doses and 2 vapor concentrations of each pair of test compounds will be utilized.

In order to determine the tissue disposition of halocarbons in two species, rats and dogs will receive equivalent exposures to halocarbons intravenously, orally and by inhalation. Concentrations of the parent compound in brain, liver, kidney, heart, lung, skeletal muscle and adipose tissue will be measured at selected intervals over time, in order to provide an assessment of the actual target organ dose for validation of physiologically-based model development and inter-species correlations with toxicity. A second series of tissue disposition experiments will be conducted to determine what adjustments in administered dose are necessary to achieve equal brain levels of test compounds in each species. An inhalation and oral exposure concentration will be administered to the rat that yields a brain level of halocarbon similar to that seen in the previous experiments in the dog.

Physiologically-based pharmacokinetic models will be developed and validated for oral and inhalation exposures to halocarbons, which will allow accurate prediction of the concentration of halocarbons in blood and tissues over time during and following exposure. Data from the direct measurements of blood and tissue concentrations will be compared

to simulated values calculated from mass-balance differential equations comprising the model. Thereby, the accuracy of the model can be tested and adjustments made where necessary to improve the model simulations. Models for inhalation and oral exposures validated using pharmacokinetic data in rats will be employed to predict blood and tissue halocarbon concentrations in the dog. The accuracy of the model for this interspecies extrapolation will be assessed by comparing the predicted concentrations to values determined experimentally for the dog.

The neurobehavioral toxicity of inhaled solvents will be correlated with the target organ concentration in the two species. Rats and dogs will be exposed to selected halocarbons at defined inhaled concentrations and lengths of exposure. Two neurobehavioral tests, one for operant performance and one for acute motor performance, will be performed periodically during and/or after exposures. The magnitude of CNS effects of each solvent will be correlated with the target organ (i.e. brain) concentration, as determined in the tissue uptake studies, at each time-point. In this manner, the feasibility of toxicity extrapolations between species based on a common target organ dose will be evaluated.

Toxicodynamic models will then be developed and validated for inhaled halocarbons. To develop this model, rat brain halocarbon concentrations will be correlated with the magnitude of neurobehavioral effects in an appropriate equation and the validity of the equation tested by comparison with actual experimental values obtained from previous stages of the project. By incorporating predicted brain concentrations from the previously validated physiologically-based pharmacokinetic model, a combined physiological pharmacokinetic-toxicodynamic model can be developed. This combined model may allow the prediction of toxicity from the interspecies extrapolation of pharmacokinetic data, and in simulations in the absence of experimental data.

II. INVESTIGATION OF THE RELATIVE ROLE OF METABOLISM IN THE PHARMACOKINETICS OF INHALED HALOCARBONS.

One of the major objectives was to fully characterize the pharmacokinetics of inhaled halocarbons during and following exposures, in order to provide a pharmacokinetic data base for interspecies comparisons and for formulation of physiologically-based pharmacokinetic models. These studies have been conducted with the Sprague-Dawley rat. These comprehensive experiments will provide the information necessary for the most efficacious design of experimental protocols for the dog. This will aid in avoiding inefficient or redundant work in the dog experiments, an important factor in view of the high cost of conducting experiments in dogs. Concentrations of the parent compounds were monitored in the blood and in the exhaled breath for appropriate periods during and after exposures in order to delineate uptake and elimination of the test chemicals. These data were then subjected to pharmacokinetic analyses and subsequently used in formulation of physiologically-based computer simulation models.

A major route of elimination of halocarbons is hepatic metabolism. If metabolism plays a significant role in the disposition and subsequent neurobehavioral effects of these chemicals, extensively metabolized halocarbons should be more rapidly eliminated (and have a less pronounced CNS depressant action, as determined in subsequent experiments than poorly metabolized halocarbons. In order to test this PREMISE, halocarbons with widely differing propensity for metabolism were studied. Extensively metabolized trichloroethylene (TCE) and dichloroethylene (DCE) and poorly metabolized 1,1,1-trichloroethane (TRI) were used. DCE and TRI are of comparable volatility, so they would be expected to be eliminated similarly by the lung. Differences in pharmacokinetics could therefore more likely be attributed to differences in metabolism. While TCE and TRI are very similar structurally (differing only in a single double bond), differences in both volatility and metabolism should be reflected in the resulting uptake and elimination of the test chemicals.

For these inhalation exposures, the halocarbon was administered to unanesthetized male Sprague-Dawley rats previously prepared with an indwelling carotid artery cannula. These rats, weighing 325-375 g, inhaled the compound for 2 hr through a one-way breathing valve in an inhalation exposure system previously developed by this laboratory. Repetitive samples of the separate inhaled and exhaled breath streams, as well as arterial blood, were collected concurrently and analyzed for the test compound. Respiratory rates and volumes were continuously monitored during and following exposure, and were used in conjunction with the pharmacokinetic data to characterize profiles of uptake and elimination. This experimental protocol has provided a unique approach by combining direct measurements of the halocarbons TCE, TRI, and DCE in the exhaled breath and blood simultaneously with detailed measurements of respiration. The separation of the inhaled and exhaled breath streams by use of the one-way breathing valve afforded both sampling of the exhaled breath for halocarbon during and following exposure and measurement of the air flow in the breath stream. The breathing valve has been used previously for monitoring respiration in unanesthetized animals (Mauderly et al., 1979), but pharmacokinetic measurements were not made using this system. In previously reported pharmacokinetic studies of inhaled halocarbons in laboratory animals, direct determinations of the exhalation of the solvent by individual animals during exposures were not made, as most of these studies employed dynamic or closed exposure chambers. Emphasis on the pharmacokinetic measurements of these halocarbons has focused primarily on measurements following the termination of exposure. Also, parameters of respiration were not monitored in these experiments. Accurate determination of the total amount of chemical absorbed or eliminated by inhalation requires monitoring of respiratory parameters. In the present study, measurement of halocarbon uptake was accomplished by calculation from either the blood level data or the exhaled breath data in conjunction with the monitored respiratory parameters.

III. STUDIES OF THE PHARMACOKINETICS OF INHALED TRI IN RATS

Studies of the pharmacokinetics of TRI in rats during and following inhalation exposure have been completed, and the results compiled in a manuscript that has been submitted for publication to a respected peer-reviewed scientific journal. The manuscript is included as Section A of the Appendix (and listed in Section D), and the reference is as follows:

> Dallas, C. E., Ramanathan, R., Muralidhara, S., Gallo, J.M. and Bruckner, J. V. "The uptake and elimination of 1,1,1-trichloroethane (TRI) during and following inhalation exposures in rats. Submitted to Toxicology and Applied Pharmacology (1988).

It was found that TRI was very rapidly absorbed from the lung, in that substantial levels were present in arterial blood at the first sampling time (i.e., 2 min). TRI blood and exhaled breath levels increased rapidly after the initiation of exposure to near steady-state within approximately 20-45 min and were then directly proportional to the exposure concentration. Percent uptake decreased over time during inhalation exposures until equilibrium was established by 1 hr, after which % uptake of the inhaled dose was approximately 50% for both exposure groups. Total cumulative uptake of 50 and 500 ppm TRI over the 2-hr inhalation exposures was determined to be 6 and 48 mg/kg bw, respectively. By the end of the exposure period, 52.5 and 56.3% of the total inhaled dose was eliminated in the breath of the low and high dose groups, respectively. A physiological pharmacokinetic model for TRI inhalation was utilized to predict blood and exhaled breath concentrations for comparison to observed experimental values. Overall, values predicted by the physiological pharmacokinetic model for TRI levels in the blood and exhaled breath were in close agreement with measured values both during and following TRI inhalation. While TRI exhaled breath levels in rats in this study were comparable to those measured previously in humans, blood levels of TRI were not equivalent in rats and man. It was therefore concluded that the rat may be a potential useful model for TRI respiratory elimination in man.

IV. STUDIES OF THE PHARMACOKINETICS OF TCE FROM INHALATION EXPOSURES IN RATS

A second manuscript on the pharmacokinetics of inhaled halocarbons has been completed, and it describes the uptake and disposition of trichloroethylene (TCE) in rats during and following inhalation exposure. This manuscript will be submitted to a peer-reviewed scientific journal simultaneously with the submission of this report to AFOSR. The manuscript is included as Section B of the Appendix (and listed in Section D), and the reference is as follows:

> Dallas, C. E., Ramanathan, R., Muralidhara, S., Gallo, J. M. and Bruckner, J. V. "Direct Measurement of trichloroethylene (TCE) in the blood and exhaled breath of rats during and following inhalation exposures". To be submitted to Fundamental and Applied Toxicology (1988).

In this study, the effect of the saturation of the metabolism of TCE during inhalation exposures on the subsequent pharmacokinetics of the compound was evaluated. TCE exhaled breath levels were found to have increased rapidly after the initiation of exposure to near steady-state within approximately 20-30 min and were then directly proportional to the exposure concentration. Uptake of TCE in the blood was also rapid, but blood levels continued to increase progressively over the course of the inhalation exposure at both dose levels. Arterial TCE concentrations were not proportional to the inhalation concentration, with levels for the 500 ppm group from 25-30 times greater than in 50 ppm-exposed rats during the second hour of the exposure. Percent uptake was nearly complete at the initiation of inhalation exposures and decreased rapidly thereafter until equilibrium was established by 1 hr, after which % uptake of the inhaled dose was approximately 69-72% for both exposure groups. Total cumulative uptake of 50 to 500 ppm TRI over the 2-hr inhalation exposures was determined to be 8.4 and 73.3 mg/kg bw, respectively. The direct measurements of TCE in the blood and exhaled breath were utilized in the validation of a physiological pharmacokinetic model for the prediction of the pharmacokinetics of inhaled TCE. Results from this study indicate that metabolism of TCE is saturable between 50 and 500 ppm exposure in rats, resulting in disproportionately higher blood levels above the saturation point. At doses below this metabolism saturation point in rats, blood and exhaled breath levels of TCE in rats were very similar to values previously published for TCE inhalation exposures in humans.

V. STUDIES OF THE PHARMACOKINETICS OF INHALED DCE IN RATS

An investigation of the uptake, disposition, and elimination of 1,1-dichloroethylene (DCE) has also been completed for inhalation and oral exposures in rats. These results were presented at the most recent meeting of the Society of Toxicology in March, 1988. The results for both the inhalation and oral studies of DCE are being prepared for manuscript submission to a peer-reviewed journal this fall. The reference for these studies (also listed in Section D) as presented in the abstract is as follows:

> Dallas, C. E., Ramanathan, R., Muralidhara, S., Gallo, J. M. and Bruckner, J. V. "Comparative pharmacokinetics of inhaled and ingested 1,1-dichloroethylene (DCE) in rats". 27th Annual Meeting of the Society of Toxicology, Dallas, TX; Toxicologist 8: 139 (1988).

The DCE inhalation studies were performed with a similar experimental protocol as was employed in the inhalation studies of TRI and TCE. However, the inhalation exposure concentrations employed were 100 and 300 ppm. The 300 ppm level (rather than 500 ppm as employed previously for TCE and TRI was deemed appropriate due to the high level of hepatotoxicity and subsequent mortality observed in rats at levels very much in excess of 300 ppm inhalation. While 100 and 300 ppm were the target DCE inhalation concentrations, the actual concentration inhaled by the animals was determined by measurements of air samples taken from the airway immediately adjacent to the breathing valve.

Inhaled DCE concentrations for the six rats in each group were 310.0 ± 3.5 ppm for the 500 ppm exposures and 101.6 ± 0.8 ppm for the 50 ppm exposures. The respiration of each animal was continuously monitored. The respiratory monitoring technique was conducted according to the methods previously published in solvent exposure studies by this laboratory (Dallas et al., 1983 and 1986). The airflow created by the animal's inspiration was recorded both during and following DCE inhalation exposure in terms of minute volume (volume of respiration per minute, or V_E), respiratory rate (f), and tidal volume (V_T). An average value for these parameters for each individual animal was obtained by averaging the measurements taken at 10-min intervals during the 2-hr exposure. The mean ± SE of these average values for the 100 ppm exposure group (n=6) were: V_E = 229.5 ± 8.9 ml/min; f = 136.2 ± 2.9 breaths/min; and V_T = 1.72 ± 0.12 ml. The mean ± SE of these average values for the 300 ppm exposure group (n=6) were: V_E = 215.7 ± 18.8 ml/min; f= 134.5 ± 11.5 breaths/min; and V_T = 1.60 ± 0.09 ml.

During and following DCE inhalation, concentrations of the parent compound were measured in the blood (Fig. 1) and exhaled breath (Fig. 2) of 100 ppm and 300 ppm exposed rats. Significant respiratory elimination of unchanged DCE was evident during the inhalation exposure period, with steady-state DCE levels achieved in the exhaled breath within 20 min at both dose levels. DCE respiratory elimination was proportional to the inhaled concentration during exposure, as indicated by the exhaled breath values during 30-120 min of the exposure period (near steady-state) of 71.6 ± 4.1 ppm and 204.6 ± 9.3 ppm (x ± SE) for the 100 and 300 ppm exposure groups, respectively. Upon cessation of DCE inhalation, the concentration of DCE declined very rapidly in the expired air of both exposure groups. Substantial concentrations of DCE were found in the blood of all animals at the first sampling time (2 min). Arterial DCE concentrations were not proportional to the inhalation concentration. After the initial rapid uptake phase over the first 20 minutes of exposure, blood levels for the 300 ppm-exposed rats were 4 to 5 times higher than DCE blood concentrations of rats that received 100 ppm exposures. The x ± SE for the blood concentrations from 30 to 120 min, during near-steady state, were 0.56 ± 0.03 and 2.19 ± 0.14 µg/ml for the 100 and 300 ppm exposure groups, respectively.

Evaluation of the ratio of DCE concentration in the blood to DCE concentration in the exhaled breath over the duration of the inhalation exposure is shown in Fig. 3. After the first few minutes of exposure, this ratio was consistently higher for the 300-ppm-exposed rats relative to the 100 ppm group, though the difference is slight until the point at the termination of exposure (120). At this point, the values are significantly different between the two dose groups. Measurement of the cumulative uptake of DCE by the rats (Fig. 4) was made by accounting for the quantity of unchanged DCE that was exhaled during the inhalation exposure period. As a result of the 2-hr exposure to 100 ppm DCE the cumulative uptake was 3.3 ± 0.3 mg (x ± SE), or 10 mg/kg bw. The total cumulative uptake of DCE from the 2-hr exposure to 300 ppm was 10.2 ± 0.6 mg (x ± SE), or 30 mg/kg bw. The amount of DCE absorbed into the systemic circulation (x^o_{abs}) reflected the relationship between the blood-concentration-time data and measured respiratory parameters. The x^o_{abs} for the 100 ppm-exposed rats was 14.3 ± 0.9 (x ± SE), or 40.9

mg/kg bw. For the 300 ppm-exposed rats the x^{o}_{abs} was 4.9 ± 0.3 mg (x SE), or 14.0 mg/kg bw. Percent uptake of DCE (inhaled-exhaled concentration/inhaled concentration) during inhalation exposure was similar for both exposure groups (Fig. 5) during the first hour of exposure. During the second hour of exposure the % uptake for the 300 ppm group is less than that of the 100 ppm-exposed rats. While % uptake for the low dose group remains relatively constant during this period, the values for the rats inhaling 300 ppm show a steady decrease.

The cumulative elimination of DCE in the exhaled breath both during and following inhalation exposure is shown in Fig. 6. During DCE exposure, the cumulative elimination is dependent on DCE in the blood and on DCE eliminated from the alveolar space that was not absorbed into the blood. The magnitude of pulmonary elimination was proportional to the inhalation exposure concentration. By the end of the 2-hr exposure to 100 and 300 ppm DCE, 2.2 ± 0.2 and 6.1 ± 1.3 mg (x ± SE), respectively, were eliminated from the rats in the exhaled breath. Following the termination of exposure, DCE that is eliminated in the breath is soley from unchanged DCE from the systemic circulation. During the 3-hr post-exposure period, an additional 0.14 and 0.37 mg of DCE were eliminated from the animals in the 100 and 300 ppm exposure groups, respectively.

The percent of the inhaled dose that was eliminated during and following the 2-hr exposures to DCE are represented as plots of percent elimination in Fig. 7. Percent elimination of 100 ppm DCE was consistently higher than that for the 300 ppm group during the inhalation exposure period. By the end of the 2-hr inhalation exposures to 100 and 300 ppm DCE, 40.0 ± 4.0 and 36.4 ± 5.3 (x ± SE), respectively, of the total inhaled dose was eliminated in the exhaled breath of exposed rats. By 3 hr post-exposure, 42.4% of the total inhaled dose of DCE had been eliminated from the 100 ppm exposed rats, while 38.7% had been eliminated in the breath of the 300 ppm exposure groups.

VI. STUDIES OF THE PHARMACOKINETICS OF INGESTED TRI, TCE, AND DCE

A variety of halocarbons and other VOCs have been identified as contaminants of food and drinking water supplies in the U.S. (Symons et al., 1975; NOMS, 1977). Some of the halocarbons most commonly identified in water supplies are 1,1-dichloroethylene (1,1-DCE), trichloroethylene (TCE), and 1,1,1-trichloroethane (TRI). As indicated previously, in addition to being important as environmental contaminants these agents have been selected for investigation in order to evaluate the relative role of the saturation of metabolism of the compounds after exposure. Emissions from product manufacturing, usage activities, and spills are thought to be primary sources of these halocarbons in water supplies. Recently, the contamination of drinking water supplies by the leakage of solvents from storage tanks and chemical waste dumps has become of significant concern. There are large numbers of solvent and fuel storage tanks in the nation, many at U.S. Air Force facilities. As the majority of these are located underground, leakage of solvents into groundwater supplies can proceed undected for years.

Despite the potential public health significance of halocarbon ingestion from contaminated drinking water supplies, there is presently insufficient information available concerning the systemic absorption and disposition of these and other halocarbons following their oral administration. Most studies have involved administration of ^{14}C-labeled halocarbons and measurement of levels of radioactivity at a single time-point following dosing, though this approach precludes delineating between paren compound and metabolites. While blood and tissue levels of halocarbons have usually been measured only 2 or 3 days after oral dosing, ingestion of certain halocarbons has been shown to result in pronounced cytotoxic effects within minutes or hours of ingestion (Moore et al., 1976; Lowrey et al., 1981; Luthra et al., 1984). Thus it is important to know the extent of systemic absorption and disposition of halocarbons in the body during the period immediately following ingestion. Therefore, investigations toward this end were initiated by this laboratory during a previous grant effort, resulting in preliminary data on ingested DCE (Putcha et al., 1986) and TCE (D'Souza et al., 1985). These studies were conducted in rats anesthetized with ether, with the compound administered with polyethylene glycol as a dosage vehicle. A major goal of these studies was to compare pharmacokinetics of the compounds in fed and fasted animals, in which it was found that food appears to delay the absorption of the halocarbons from the gut. The bioavailability of these agents was equivalent in animals given the same dose by oral or intravenous administration. Therefore, it is apparent that absorption of the orally administered halocarbon is complete.

In the present investigation, the halocarbons used as test chemicals were administered to unanesthetized rats. The disadvantages of using anesthesia during chemical exposures were thus avoided. Anesthetics such as phenobarbital and diethyl ether are known to inhibit the metabolism of drugs which are biotransformed by the hepatic mixed function oxidase system (Johannssen et al., 1981; Vermeulen et al., 1983). It is possible that use of anesthesia may alter the metabolism and kinetics of halocarbons during exposure. Accordingly, we utilized an unanesthetized animal model approach in order to be certain that the halocarbon pharmacokinetic results obtained were representative of kinetics in unanesthetized, relatively unstressed animals.

As anesthetics have a potential to affect respiration rates and volumes, they could alter the quantity of test chemical which is eliminated. For evaluating the oral pharmacokinetics of chemicals like halocarbons, this can be a very important factor since halocarbon elimination in the breath has been shown to be a significant factor following halocarbon ingestion (Chieco et al., 1981; Dallas et al., 1986). Increased or decreased respiration may alter the rate and magnitude of the respiratory excretion of the halocarbon, thus affecting systemic kinetics as well. Other problems such as anesthetic-induced fluctuations in body temperature would also be avoided by use of an unanesthetized animal model. Significant changes in body temperature have an impact on enzyme systems that can alter the metabolism and pharmacokinetics of certain chemicals. While core temperature can be monitored and periodically readjusted with a heating pad for anesthetized animals, some changes are inevitable with the system.

Also, possible competitive metabolic inhibition and alteration in transport processes by anesthetics are avoided in an unanesthetized model.

Studies of the dose dependence of the pharmacokinetics of ingested DCE in unanesthetized rats have been completed. These results were presented as part of the Society of Toxicology abstract previously cited on page 5. Previous studies of DCE in rats have indicated that lethality (Andersen and Jenkins, 1977) and hepatotoxicity (Andersen et al., 1979a) have abrupt increases in response over a definite range of DCE exposure concentration. Saturation of the metabolic activation of DCE is believed to occur due to depletion of glutathione, leading to this sudden increase in toxicity. This capacity of the rat to metabolize DCE may have been exceeded by a single oral dose between the range of 50 to 100 mg/kg (Andersen and Jenkins, 1977; McKenna et al., 1978). In the studies thus far completed, the pharmacokinetics of DCE in unanesthetized rats have been evaluated over a range of doses below this perceived metabolic saturation point for DCE ingestion, with additional dose studies expected to exceed DCE metabolism in the rat to be conducted later.

In order to procure repetitive blood samples following adminstration of single oral bolus doses of halocarbons to unanesthetized rats, an indwelling arterial cannula was surgically implanted prior to the halocarbon exposure. The cannula was tunneled subcutaneously to the back of the animal and exited just behind the head. The cannula was extruded through a steel spring that was attached to the back of the animal by a harness. After the surgery was complete, the animal was placed into a metabolism cage to recover for 24 hours before halocarbon dosing. The steel spring was exited through the top of the cage and connected to a counter-balance weight system, which prevented the animal from interfering with the cannula but allowed relative freedom of movement.

The rats were given the halocarbon in an aqueous Emulphor emulsion as a single oral bolus dose of 10 and 30 mg/kg DCE. Blood samples were taken from the carotid artery cannula for up to 5 hours following the oral dose. The halocarbon content of the blood samples was measured with a gas chromatograph equipped with a semi-automatic headspace sampler and an electron capture detector.

The total body clearance (CL_T) of 1,1-DCE was calculated using the formula:

$$CL_T = \frac{\text{Dose}}{\int_0^{\infty} C\,dt}$$

where $\int_0^{\infty} C\,dt$ is the area under the blood concentration versus time curve (AUC) of 1,1-DCE.

Apparent volume of distribution (V_β) was calculated using the formula:

$$V_\beta = \frac{\text{Dose}}{\beta_o \int^{oo} C dt}$$

The biological half-life ($t_{\frac{1}{2}}$) of 1,1-DCE was calculated by the formula:

$$t_{\frac{1}{2}} = \frac{0.693}{\beta}$$

where β is the terminal elimination rate constant.

The volume of distribution of the central compartment (V_c) was calculated using the formula:

$$V_c = \frac{\text{Dose}}{P+A+B}$$

where P, A and B are the 0-time intercepts of the three exponential phases of the blood concentration versus tire curves.

The blood concentration-time profiles for the oral administration of DCE in unanesthetized rats are shown in Fig. 8. DCE was very rapidly absorbed from the gut, as peak blood levels of DCE were reached within 4 minutes after oral bolus dosing. Comparison of the pharmacokinetic parameters between the two dose groups are shown in Table I. The maximum blood concentration (C-MAX) reached following 30 mg/kg dosing was 4.3 times that achieved following oral administration of 10 mg/kg. Comparing the area-under-the-blood-concentration-time curve (AUC) also indicated a disproportionate difference of 4.7 times between the two dose groups. The elimination half-lives ($t_{\frac{1}{2}}$) were similar at the two dose levels, however. Both the apparent clearance and volume of distribution of the 30 mg/kg dose group were approximately two-thirds that of the 10 mg/kg group. Compared to the previous data from the oral administration of DCE in anesthetized rats by this laboratory (Putcha et al., 1986), the terminal elimination $t_{\frac{1}{2}}$ and AUC were lower in the current investigation with unanesthetized rats. Using a 10 mg/kg dose in both studies as an example, the mean $t_{\frac{1}{2}}$ and AUC values for the anesthetized rats were 181 µg-min/ml 78.2 minutes, respectively, while these two parameters for unanesthetized rats were 50.5 µg-min/ml and 50 minutes, respectively. These differences, however, might not be attributable only to the use of anesthesia. The previous study with anesthetized rats also employed 50% aqueous polyethylene glycol (PEG) 400 as a diluent, while an aqueous emulsion (emulphor) was used in the present investigation.

The investigation of the pharmacokinetics of TRI following oral administration as a single bolus in unanesthetized rats has also been completed. These results were presented at the most recent meeting of the Society of Toxicology in Dallas, Texas. It is intended that this study will be combined with the results of experiments currently underway involving the intravenous administration of TRI for the submission of a manuscript to a peer-reviewed journal by the end of the

year. The reference for the study of orally-administered TRI (also listed in Section D of the Appendix) as presented in the abstract is as follows:

> Muralidhara, S., Ramanathan, R., Gallo, J. M., Dallas, C. E., and Bruckner, J. V. "Pharmacokinetics of volatile halocarbons: Comparison of single oral bolus versus gastric infusion of 1,1,1-trichloroethane (TRI)" 27th Annual Meeting of the Society of Toxicology, Dallas, TX; Toxicologist 8: 95 (1988).

The experimental protocol for this pharmacokinetic study of TRI was similar to that conducted for DCE. Due to the longer half-life of TRI relative to DCE, though, sampling for ingested TRI was conducted for longer intervals of time (up to 480 minutes after a single oral bolus administration).

Measurements of the systemic uptake, disposition, and elimination of TRI after ingestion in the unanesthetized rat is shown in Fig. 9. TRI was administered as a single oral bolus dose of either 6 or 48 mg/kg, and samples of arterial blood taken periodically from an indwelling carotid artery cannula and analyzed for TRI by gas chromatography. Samples were taken frequently in the first minutes following the oral dose in order to characterize the very rapid uptake of ingested TRI into the systemic circulation. Arterial blood levels reached a peak 8 to 12 minutes after oral dosing and declined relatively quickly thereafter. The pharmacokinetic parameters for the two dose groups are shown in Table II. There was a distinct linear relationship between the maximum blood concentration (C-MAX) achieved from the two doses employed. The AUC values were also approximately proportionate to the administered dose. As was seen for DCE previously, the elimination half-life of TRI was nearly identical for both dose groups (112-115 minutes). Even though DCE and TRI have a similar characteristic volatility (blood:air partition coefficient near 5), the disappearance of ingested DCE from the blood occurs more than twice as fast as does the disappearance of ingested TRI. This can be attributed to the high rate of metabolism of DCE relative to poorly metabolized TRI.

Studies of the pharmacokinetics of ingested TCE have also been conducted. Results from these experiments were also presented at the most recent meeting of the Society of Toxicology earlier this year. The reference for this study (also listed in Section D of the Appendix) as presented in the abstract is as follows:

> Ramanathan, R., Muralidhara, S., Gallo, J. M., Dallas, C. E., and Bruckner, J. V. "Pharmacokinetics of volatile halocarbons: Comparison of single oral bolus versus infusion of trichloroethylene (TCE)". 27th Annual Meeting of the Society of Toxicology, Dallas, TX; Toxicologist 8: 94 (1988).

The same procedure was employed for TCE determinations after oral exposure as were used previously for DCE and TRI. A single oral bolus of 8 and 76 mg/kg TCE was administered to unanesthetized rats with an indwelling carotid arterial cannula.

The TCE blood concentration-time profile following oral administration is shown in Figure 10. As was previously observed with DCE and TRI, blood levels of TCE rose quickly after oral dosing to indicate rapid absorption from the gastrointestinal tract. Peak arterial blood levels were reached 8-12 minutes after the single oral bolus dose. The pharmacokinetic parameters for the oral administration of TCE are shown in Table III. The AUC for the 76 mg/kg group was 22 times that seen after 8 mg/kg. Due to the known capacity for TCE to saturate the metabolism of the rat with a sufficient dose, this is an indicator that TCE metabolism is possibly saturable for a single oral bolus dose between 8 and 76 mg/kg. Unlike DCE and TRI, which each had similar elimination half-lives ($t_{\frac{1}{2}}$) for dose levels of 3 and 8 fold-difference in magnitude, respectively, the $t_{\frac{1}{2}}$ of TCE was significantly different for the two dose groups. The 76 mg/kg group demonstrated an approximate 50% increase in $t_{\frac{1}{2}}$ relative to the 8 mg/kg group.

VII. DEVELOPMENT AND VALIDATION OF PHYSIOLOGICALLY-BASED MODELS IN THE PREDICTION OF HALOCARBON PHARMACOKINETICS IN THE RAT

An important goal of the project has been to develop and validate physiologically-based pharmacokinetic (PBPK) models, which will allow accurate prediction of the concentration of halocarbons in blood and tissues over time following inhalation and oral exposure. The pharmacokinetic studies conducted in earlier phases of the project have thus provided a unique data base from which to formulate and test the models. Data from the direct measurements of blood and exhaled breath levels of halocarbon have been compared to simulated values calculated from mass-balance differential equations comprising the model. Thereby, the accuracy of the model has been tested by comparison to observed blood and exhaled breath concentrations.

The current investigation of the uptake and elimination of TRI in rats provided the first available data base for direct measurement of TRI in the exhaled breath and blood during inhalation exposures in rats (See Appendix A, Figures 2 and 3). A PBPK model was therefore developed to describe the disposition of TRI in the rat (Appendix A, Figure 1) using this unique opportunity for comparison of computer simulated values with these direct measurements for validation of the model. The fundamental characterisitics of this initial model development were based on the work by Ramsey and Andersen (1984) and their PBPK model for the prediction of the kinetics of inhaled styrene. Model-generated simulations of blood and fat styrene concentrations were in agreement with concentrations measured over a period of hours in rats subjected to a series of vapor levels of styrene. Andersen et al. (1984) expanded their inhalation model for brominated dihalomethanes to forecast not only the time-course of the parent chemical, but the time-course of two metabolites as well.

In the present model development for TRI, it was assumed that a blood flow-limited model was adequate to characterize the tissue distribution of TRI. Blood flow-limited tissue compartments consider the chemical to be homogeneously distributed throughout the blood,

interstitial and intracellular spaces. The differential mass balance equation used for a non-eliminating blood flow-limited organ was:

$$V_i \frac{dC_i}{dt} = Q_i (C_B - \frac{C_i}{R_i})$$

where: V_i = volume of the i^{th} organ
C_i = concentration in the i^{th} organ
Q_i = blood flow for i^{th} organ
C_B = blood concentration
R_i = partition coefficient

Eliminating organs, such as the liver, required a clearance term added to this equation for blood flow-limited compartments. In the case where clearance was constant, the term: $-CL_i C_i/R_i$ would be employed where CL_i is equal to the intrinsic clearance for the i^{th} organ. For nonlinear clearance, the term $-V_m C_i/(K_m+C_i)$ was added to the appropriate mass balance equation. V_m equals the maximum rate of the elimination process (i.e., metabolism) and K_m equals the concentration at which the rate is half the maximum value.

For TRI, compartmental volumes and organ blood flows were obtained from Ramsey and Andersen (1984) and scaled to 340 g, the mean body weight of rats used in the present study. Partition coefficients for TRI were taken from Gargas et al. (1986). Total systemic clearance was obtained from a preliminary study in rats. In this study, 50 mg/kg of TRI was administered as a 2-hr constant rate iv infusion with blood samples collected for up to 9 hr after the termination of the infusion (data not shown). The total systemic clearance was calculated as the dose divided by the total area under the TRI blood concentration-time curve. Differential mass balance equations, incorporating the parameters listed in Table 1 of Appendix A, that described the transport of TRI in the rat were solved with the ACSL, Advanced Continuous Simulation Language, computer program (Mitchell and Gauthier, Concord, MA). The solution to the equations provided predicted TRI concentrations over time.

The predicted values for the elimination of TRI in the exhaled breath were in very close agreement with the measured values determined in the inhalation exposure to TRI in rats reported in this project (Appendix A, Figure 2 and 3). The simulations were close to the experimentally-observed values during both the uptake and steady-state phases during TRI inhalation, and in the elimination phase after the termination of exposure. The concentration-time profiles of TRI in the blood were also well-described by the PBPK model. Only for the near steady-state phase during inhalation exposure to 50 ppm TRI was there a relatively small overestimation (about 50 ng/ml) of the predicted blood levels relative to the observed blood levels. The ability of the model to accurately predict TRI levels in samples from two different physiological sources in the rat was encouraging in this initial model development effort.

In order to develop a PBPK model in which saturable metabolism is a factor, the simulation of TCE concentrations during and following

inhalation exposure were made. The model developed for inhaled TCE pharmacokinetics is shown in Figure 1 of Appendix B. A comparison of the observed experimental values and the model simulations for inhaled TCE uptake and elimination are shown in Figures 2 and 3 of Appendix B.

In general the predictions are in agreement with the actual data, with the model overpredicting blood concentrations during the 50 ppm exposure and the exhaled breath concentration during the 500 ppm exposure. These overpredictions are on the order of 0.1 µg/ml. From 180 min, all predicted values are in excellent agreement with the observed TCE concentrations. The present model incorporated the dynamics between the venous, alveolar and arterial compartments that have been used for a methylene chloride PBPK model (Angelo and Pritchard, 1984, 1987). This representation is appealing in that venous and arterial blood pools are distinct, and a physiologically realistic membrane transport term (h) controls chemical uptake and elimination at the alveolar-lung interface. The blood flow-limited tissue compartments and the Michelis-Menten liver elimination are similar to other models on the metabolized volatile organic compounds (Andersen et al., 1987). The experimentally measured model parameters, Va and the inhaled gas concentration, were the only values that were altered for the predictions obtained at the 50 and 500 ppm exposures. Tissue TCE concentration would be of great use in potential revisions of the model and in validating it.

The PBPK model developed in the previous studies for inhaled TRI has also been evaluated for its utility in predicting halocarbon pharmacokinetics following oral administration. The differential mass balance equations comprising the model included the parameters for compartmental volumes, organ blood flows, and partition coefficients as listed in Table I of Appendix A. Input into the model was obtained from the absorption rate-constant controlling TRI uptake into the systemic circulation, and was determined from analysis of blood concentration-time data. Simulated values of the uptake and elimination of a single oral dose of 6 mg/kg of TRI are presented in Figure 11, along with the observed experimental values in rats from the current investigation. Observed and simulated values for TRI oral exposures of 48 mg/kg are shown in Figure 12. An absolute bioavailability (F) of 0.5 and a Ka of 0.05 was employed in these simulations of TRI ingestion. A comparison of these observed and simulated values reveals a reasonable similarity in concentration in the uptake and elimination of ingested TRI. C-MAX, or maximum concentration of TRI attained following ingestion of the compound of the computer simulation, was very close in magnitude to the observed peak arterial concentration observed at both dose levels employed.

III. STATEMENT OF SIGNIFICANCE OF THE STUDIES FOR THE FIRST YEAR

Comparison of the pharmacokinetic results for the inhalation of TRI, DCE, and TCE in rats provides insight into the relative importance of the roles of metabolism and volatility in the uptake, disposition, and elimination of these halocarbons. Detailed discussions of the results are presented in the manuscripts submitted for publication and included with this report for TRI (Appendix A) and TCE (Appendix B).

DCE and TRI have a similar propensity for volatility as reflected by blood:air partition coefficients of 5.0 and 5.8. However, the two halocarbons are quite different in their propensity for metabolism. Studies of inhaled DCE excretion patterns have indicated that sufficiently high doses exceed the metabolic capacity of exposed animals (Andersen and Jenkins, 1977; McKenna et al., 1978; Jones and Hathaway, 1978). Pharmacokinetic studies of TRI in rats (Schumann et al., 1982) and humans (Nolan et al., 1984), though, have clearly demonstrated that this halocarbon is not metabolized to a significant extent in either species. Indeed, with the high volatility and relative lack of metabolism, the majority of the inhaled TRI was found to be eliminated unchanged in the breath of rats and man in a linear pattern related to dose. In the present study in rats, DCE and TRI were found to have a very similar magnitude of uptake of the inhaled dose over time (adjusted for differences in inhalation concentration). For instance, DCE uptake for 100 ppm exposure for 2 hours was 10 mg/kg, while TRI uptake at half that dose (50 ppm exposure) for 2 hours was 6 mg/kg. By contrast, total uptake of TCE for a 50 ppm exposure for 2 hours was 8.4 mg/kg. Like DCE, TCE has been found to be significantly metabolized to various metabolites in rats (Stott et al., 1982). For the inhalation of TCE, this dose-dependent metabolism was calculated to be saturable at approximately 65 ppm (Filser and Bolt, 1979). Unlike DCE and TRI, however, TCE has a relatively high blood:air partition coefficient (21.9), which indicates that volatility is less of a factor for TCE relative to these halocarbons. As DCE and TRI uptake were similar and both were lower than for TCE, it seems that the characteristic volatility of the halocarbon is relatively more significant to the total uptake during inhalation exposures than the role of metabolism.

Metabolism was a key factor, however, when considering the systemic disposition of inhaled halocarbons as reflected by the blood levels of inhaled TCE, TRI, and DCE. For all three halocarbons, substantial levels of the inhaled compounds were present in the blood within minutes after the initiation of exposure. Each inhaled halocarbon demonstrated a rapid uptake phase that encompassed approximately the first thirty minutes of inhalation exposure. Once a near steady-state was achieved after this rapid uptake phase, however, a distinct pharmacokinetic difference exists between the three halocarbons related to the propensity for metabolism by the compound. Near-steady-state levels for TCE and DCE were not proportional to the inhaled concentration, while steady-state levels for inhaled TRI were proportional to the inhalation exposure level. Indeed, TCE blood levels continued to increase progressively throughout the inhalation of 500 ppm TCE, and were 25-30 times greater than in 50 ppm-exposed rats. As DCE and TCE are significantly metabolized and TRI is not, this indicates that metabolism (and the saturation of metabolic capacity) is still a very important factor in systemic disposition patterns of inhaled halocarbons.

In view of the aforementioned relationships, an interesting finding in the present investigation is the data involving the measurement of the respiratory elimination of the halocarbons in the rat. As in the determinations of halocarbon uptake in the blood, elimination of TCE, TRI, and DCE in the breath increased rapidly during the first thirty minutes of exposure until a near steady state equilibrium was reached thereafter. Unlike uptake in the blood, however, near steady-state

exhaled breath levels of all three halocarbons were proportional to the inhalation exposure concentration. This is not surprising when considering TRI, a halocarbon which does not undergo significant metabolism and also exhibits linear pharmacokinetics in systemic uptake in the blood. For both DCE and TCE, though, respiratory elimination of these well-metabolized halocarbons remained linear to the inhaled dose regardless of disproportionate uptake occurring simultaneously in the blood. It is apparent that the characteristic high volatility of halocarbons is a critical factor in determining the relative elimination of the compounds in the rat, at least in short-term inhalation exposures.

Determinations of elimination of halocarbons in the breath of rats has also revealed a finding that is of significance to the interspecies extrapolation of pharmacokinetic data from rats to humans. In addition to their structural similarity yet definitive differences in metabolism, TCE and TRI were selected as test chemicals in the present investigation because there is a unique pharmacokinetic data base available for these two halocarbons in humans. Therefore, a comparison of the previously unavailable direct measurements in the rat in the present investigation could be made with these pharmacokinetic determinations conducted in humans. A very interesting finding from this comparison is that the concentration of TCE and TRI in the exhaled breath of rats was very similar to that measured in humans, adjusting for differences in the exposure concentration employed. For TRI, there was exhaled breath data available during inhalation exposures in humans (Nolan et al., 1984), as well as following exposure. The exhaled breath levels after 1.5 hr of exposure to 35 and 350 ppm of TRI were 0.14 and 1.28 µg/ml, respectively. Assuming a linear scale-up to a 50 and 500 ppm exposure (0.2 and 1.83 µg/ml, respectively), these exhaled breath levels in humans are very similar to exhaled levels measured after 1.5 hr of exposure in the present study in rats (0.21 and 2.16 µg/ml, respectively). In both the human study and the rat study reported here, TRI elimination in the breath was proportional to the exposure concentration.

Exhaled breath determinations of TCE in humans have centered on measurements conducted following the termination of exposure. The exhaled breath of exposed workers has been monitored for expired TCE following inhalation exposure as a non-invasive method for indicating the magnitude of prior exposure to the solvent (Stewart et al., 1970, 1974). Measurements of TCE in both the blood and exhaled breath of workers following TCE inhalation have been made in studies of the effect of workload (Monster et al., 1976) and repeated exposure to TCE (Monster et al., 1979) on subsequent pharmacokinetics of the inhaled solvent. Accounting for differences in exposure concentration, the post-exposure exhaled breath levels of TCE from these studies in humans were similar in magnitude to the values of TCE eliminated in the exhaled breath of rats following inhalation exposure in the current investigation. For instance, Stewart et al. (1974) found human exhaled TCE levels of 0.70 and 0.28 ppm at 30 and 120 min, respectively, after termination of 20 ppm TCE inhalation for 3 hrs. Scaling-down the 50 ppm data in rats in the present study at these time points would yield 0.92 and 0.28 ppm, respectively. Apparently due to its relatively high hepatotoxicity, even after brief inhalation exposure in animals, there have not been

pharmacokinetic determinations of DCE made in humans. It is evident that with halocarbons for which volatility is such a critically important characteristic, such as TCE and TRI, elimination in the breath of rats and man follows a similar pattern. These results indicate that the rat may be a potentially useful model for evaluating the respiratory elimination of inhaled volatile halocarbons in man.

Similarities in the anatomy and physiology of mammalian species make interspecies extrapolations (i.e. animal scale-up) possible (Dedrick, 1973); Boxenbaum, 1984). A model developed in one species may be scaled to allow prediction of chemical concentrations in other species without the collection of additional experimental data. Model parameters such as tissue volumes and blood flows can be scaled based on allometric relationships which are functions of animal weight. The ability to scale physiological models validated in animals to humans is a powerful tool to obtain predictions of tissue chemical concentrations in humans.

Confidence in the use of scaled animal-based models to predict the kinetics of chemicals in humans can be gained only if the animal model can accurately predict chemical concentrations measured in the animal. The model utilized in the present investigation predicted blood and exhaled breath concentrations of TRI and TCE reasonably well during and following inhalation exposures in the rat, as determined by comparison with direct measurements conducted in studies on this project. The same model was able to predict peak blood levels and the systemic elimination of TRI following oral exposure with reasonable accuracy, indicating potential utility of the approach for the simulation of the ingestion of halocarbons. However, the model will require further evaluation before it can serve as a reliable predictor for halocarbon tissue concentrations. To this end, measurement of rat tissue concentrations of TRI and TCE will be necessary for additional refinements and verification of the present model. Subsequent pharmacokinetic studies in the dogs, to be initiated in the second year of the project, will then be used to further develop and validate this PBPK model for the interspecies scale-up of pharmacokinetic data. It is anticipated that a model thus validated will have the following important applications: (a) prediction of blood and target organ levels following inhalation and ingestion of halocarbons, in the absence of data; (b) interspecies extrapolations (i.e. scale-up from small to large laboratory animals and ultimately to man).

One of the benefits from the completion of these studies of TCE and TRI in the rat during the first year of this project is that there is a unique data base with pharmacokinetic determinations of the uptake and elimination of these two halocarbons in humans. Therefore, the PBPK model validated for interspecies scale-up from the rat to the dog in experiments in this project can then be evaluated for its ability to extrapolate from either of these species to man, using comparisons to these previously published values for human halocarbon pharmacokinetics. Such a validation would establish greater merit in employing the established model in making extrapolations of pharmacokinetic data from test animal species to man, or to predict blood and tissue levels of halocarbons in man in the absence of experimental data.

IX. COLLABORATIVE ARRANGEMENTS

As Principal Investigator, Dr. Cham E. Dallas has been responsible for the overall supervision of the project during the first year. Dr. Dallas has personally conducted all of the inhalation exposures, including experiments with TCE, TRI, DCE, and PER. He has also developed the novel mathematical approaches to the analysis of the respiratory monitoring data for the halocarbon inhalation studies (see Appendix A). Dr. James V. Bruckner, as Co-Principal Investigator, has been responsible for the design and conduct of the pharmacokinetic studies of halocarbon ingestion. Dr. James Gallo has had the primary responsibility for the development and validation of the physiologically-based pharmacokinetic model from the experimental studies. Dr. R. Ramanathan has participated in the analysis of blood samples for halocarbon uptake and disposition from the test animals. This effort was in conjunction with an EPA project on the effect of exposure route on the toxicity of volatile organics, which is intended for use in setting drinking water standards. Mr. S. Muralidhara has conducted the animal surgery required, analytical determinations of blood samples, oral exposures to halocarbons, and data analysis. Mr. Kevin Koporec and Miss Elizabeth Lehman are a graduate toxicology student and undergraduate chemistry student, respectively, who have assisted in the conduct of the laboratory studies, glassware washing and record keeping. Very useful technical information and counsel on the development of the PBPK models and the analysis of pharmacokinetic data in halocarbon inhalation exposures has been received in consultation with Drs. Melvin Andersen, Harvey Clewell, and Michael Gargas at the Biochemical Toxicology Branch, Toxic Hazards Division, Harry G. Armstrong Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base.

REFERENCES

Andersen, M. E., Archer, R. L., Clewell, H. J., and MacNaughton, M. G.: A physiological model of the intravenous and inhalation pharmacokinetics of three dihalomethanes - CH_2Cl_2, CH_2BrCl, and CH_2Br_2 - in the rat. Toxicologist 4: 111 (1984).

Andersen, M. E., Gargas, M. L., Clewell, H. J. and Severyn, K. M.: Quantitative evaluation of the metabolic interactions between trichloroethylene and 1,1-dichloroethylene in vivo using gas uptake methods. Toxicol. Appl. Pharmacol. 89: 149-157 (1987).

Andersen, M. E., Gargas, M. L., Jones, R. A. and Jenkins, L. J., Jr.: The use of inhalation techniques to assess the kinetic constants of 1,1-dichloroethylene metabolism. Toxicol. Appl. Pharmacol 47: 395-409 (1979).

Andersen, M. E. and Jenkins, L. J., Jr.: Oral toxicity of 1,1-dichloroethylene in the rat: Effects of sex, age, and fasting. Environ. Health Perspec. 21: 157-163 (1977).

Angelo, M. J. and Pritchard, A. B.: Simulations of methylene chloride pharmacokinetics using a physiologically based model. Reg. Toxicol. Pharmacol. 4: 329-339 (1984).

Angelo, M. J. and Pritchard, A. B.: Route-to-Route extrapolation of dichloromethane exposure using a physiological pharmacokinetic model. In Pharmacokinetics in Risk Assessment, Drinking Water and Health, Vol. 8, National Academy Press, Washington, DC.

Boxenbaum, H.: Interspecies pharmacokinetic scaling and the evolutionary-comparative paradigm. Drug Metab. Rev. 15: 1071-1121 (1984).

Chieco, P., Moslen, M. T. and Reynolds, E. S.: Effect of administrative vehicle on oral 1,1-dichloroethylene toxicity. Toxicol. Appl. Pharmacol. 57: 146-155 (1981).

Dallas, C. E., Weir, F. W., Feldman, S., Putcha, L. and Bruckner,J. V.: "The uptake and disposition of 1,1-dichloroethylene in rats during inhalation exposure". Toxicol. Appl. Pharmacol. 68: 140-151 (1983).

Dallas, C. E., Bruckner, J. V., Maedgen, J. L. and Weir, F. W.: "A method for direct measurement of systemic uptake and elimination of volatile organics in small animals." J. Pharmacol. Methods 16: 239-250 (1986).

Dedrick, R. L.: Animal scale-up. J. Pharmacokinet. Biopharm. 1: 435-461 (1973).

D'Souza, R., Bruckner, J. V. and Feldman, S.: Oral and intravenous trichloroethylene pharmacokinetics in the rat. J. Toxicol. Environ. Health 15: 587-601 (1985).

Filser, J. G. and Bolt, H. M.: Pharmacokinetics of halogenated ethylenes in the rat. Arch. Toxicol. 42: 123-136 (1979).

Gargas, M. L., Andersen, M. E. and Clewell, H. J., III: A physiologically-based simulation approach for determining metabolic constants from gas uptake data. Toxicol. Appl. Pharmacol. 86: 341-352 (1986).

Johannessen, W., Gadeholt, G., and Aarbakke, J.: Effect of diethyl ether anesthesia on the pharmacokinetics of antipyrine and paracetamol in the rat. J. Pharm. Pharmacol. 33: 365-368 (1981).

Jones, B. K. and Hathway, D. E.: The biological fate of vinylidene chloride in rats. Chem. Biol. Interactions 20: 27-41 (1978).

Lowrey, K., Glenda, E. A. and Recknagel, R.: Rapid depression of rat liver microsomal calcium pump activity after administration of carbon tetrachloride or bromotrichloromethane and lack of effect after ethanol. Toxicol. Appl. Pharmacol. 59: 389-394 (1981).

Luthra, R., Kyle, G. M., Mehta, P. S. and Bruckner, J. V.: Effects of carbon tetrachloride and 1,1-dichloroethylene on rat hepatic microsomal calcium- and/or magnesium-stimulated ATPase. Biochem. Pharmacol. 33: 3295-3298 (1984).

Mauderly, J. L., Tesarek, J. E., Sifford, L. J. and Sifford, L. J.: Respiratory measurements of unsedated small laboratory mammals using nonrebreathing valves. Lab Anim. Sci. 29: 323-329 (1979).

McKenna, M. J., Zempel, J. A., Madrid, E. O., Braun, W. H. and Gehring, P. J.: Metabolism and pharmacokinetic profile of vinylidene chloride in rats following oral administration. Toxicol. Appl. Pharmacol. 45: 821-835 (1978).

Monster, A. C., Boesma, G., Duba, W. C.: Pharmacokinetics of trichloroethylene in volunteers: Influenza of work load and exposure concentration. Int. Arch. Occup. Environ. Health 38: 87-102 (1976).

Monster, A. C., Boersma, G. and Steenweg, H.: Kinetics of 1,1,1-trichloroethane in volunteers: Influence of exposure concentration and work load. Int. Arch. Occup. Environ. Health 42: 293-302 (1979).

Moore, L., Davenport, G. D. and Landon, E. J.: Calcium uptake of a rat liver microsomal subcellular fraction in response to in vivo administration of carbon tetrachloride. J. Biol. Chem. 251: 1197-1201 (1976).

Nolan, R. J., Freshour, N. L., Rick, D. L., McCarty, L. P. and Saunders, J. H.: Kinetics and metabolism of inhaled methyl chloroform (1,1,1-trichloroethane) in male volunteers. Fund. Appl. Toxicol. 4: 654-662 (1984).

NOMS (National Organics Monitoring Survey). General review of results and methodology: Phase I-III. Office of Water Supply, U.S. Environmental Protection Agency, Washington, DC (1977).

Putcha, L., Bruckner, J. V., D'Souza, R., Desai, F. and Feldman, S.: Toxicokinetics and bioavailability of oral and intravenous 1,1-dichloroethylene. Fundam. Appl. Toxicol. 6: 240-250 (1986).

Ramsey, J. C. and Andersen, M. E.: A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. Toxicol. Appl. Pharmacol. 73: 159-175 (1984).

Schumann, A. M., Fox, T. R. and Watanabe, P. G.: ^{14}C-Methyl chloroform (1,1,1-trichloroethane): Pharmacokinetics in rats and mice following inhalation exposure. Toxicol. Appl. Pharmacol. 62: 390-401 (1982).

Stewart, R. D., Dodd, H. C., Gay, H. H. and Erley, D. S.: Experimental human exposure to trichloroethylene. Arch. Environ. Health 20: 64-71 (1970).

Stewart, R. D., Hake, C. L. and Peterson, J. E.: Use of breath analysis to monitor trichloroethylene exposures. Arch. Environ. Health 29: 6-13 (1974).

Stott, W. T., Quast, J. F. and Watanabe, P. G.: The pharmacokinetics and macromolecular interactions of trichloroethylene in mice and rats. Toxicol. Appl. Pharmacol. 62: 137-151 (1982).

Symons, J. M., Bellar, T. A., Carswell, J. K., De Marco, J., Kropp, K. L., Robeck, G. G., Seegar, D. R., Slocum, C. J., Smith, B. L. and Stevens, A. A.: National organics reconnaissance survey for halogenated organics. J. Amer. Waterworks Assoc. 67: 634-647 (1975).

Vermeulen, N. P. E., Danhof, M., Setiawan, I. and Breimer, D. D.: Disposition of hexobarbital in the rat. Estimation of "first-pass" elimination and influence of ether anesthesia. J. Pharmacol. Exper. Therap. 226: 201-205 (1983).

APPENDIX A

MANUSCRIPT SUBMITTED TO THE JOURNAL

TOXICOLOGY AND APPLIED PHARMACOLOGY

Dallas, C. E., Ramanathan, R., Muralidhara, S., Gallo, J. M. and Bruckner, J. V. "The uptake and elimination of 1,1,1-trichloroethane (TRI) during and following inhalation exposures in rats. Submitted to Toxicology and Applied Pharmacology (1988).

The Uptake and Elimination of 1,1,1-Trichloroethane (TRI) During and Following Inhalation Exposures in Rats[1,2]

CHAM E. DALLAS[3], RAGHUPATHY RAMANATHAN, SRINIVASA MURALIDHARA, JAMES M. GALLO*, AND JAMES V. BRUCKNER

Department of Pharmacology and Toxicology
*Department of Pharmaceutics
College of Pharmacy
University of Georgia
Athens, GA 30602

Abbreviated title: Kinetics of Inhaled Trichloroethane

Please send all correspondence to:

Dr. Cham E. Dallas
Department of Pharmacology and Toxicology
College of Pharmacy
University of Georgia
Athens, GA 30602

The Uptake and Elimination of 1,1,1-Trichloroethane (TRI) During and Following Inhalation Exposure in Rats. Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., and Bruckner, J.V. (1988). Toxicol. Appl. Pharmacol. 0,000-000. The pharmacokinetics of 1,1,1-trichloroethane (TRI) was studied in male Sprague-Dawley rats in order to characterize and quantify TRI uptake and elimination by direct measurements of the inhaled compound. Fifty or 500 ppm TRI was inhaled for 2 hr through a one-way breathing valve by unanesthetized rats of 325-375 g. Repetitive samples of the separate inhaled and exhaled breath streams, as well as arterial blood, were collected concurrently both during and following TRI inhalation and analyzed for TRI by gas chromatography. Respiratory rates and volumes were continuously monitored during and following exposure, and were used in conjunction with the pharmacokinetic data to characterize profiles of uptake and elimination. TRI was very rapidly absorbed from the lung, in that substantial levels were present in arterial blood at the first sampling time (i.e., 2 min). TRI blood and exhaled breath levels increased rapidly after the initiation of exposure to near steady-state within approximately 20-45 min and were then directly proportional to the exposure concentration. Percent uptake decreased over time during inhalation exposures until equilibrium was established by 1 hr, after which % uptake of the inhaled dose was approximately 50% for both exposure groups. Total cumulative uptake of 50 and 500 ppm TRI over the 2-hr inhalation exposure was determined to be 6 and 48 mg/kg bw, respectively. By the end of the exposure period, 52.5 and 56.3% of the total inhaled dose was eliminated in the breath of the low and high dose groups, respectively. A physiological pharmacokinetic model for TRI inhalation was utilized to predict blood and exhaled breath concentrations for comparison to observed experimental values.

Overall, values predicted by the physiological pharmacokinetic model for TRI levels in the blood and exhaled breath were in close agreement with measured values both during and following TRI inhalation. As the measured exhaled breath levels of TRI in rats are very similar to previously published values in humans, the rat may be a potentially useful model for TRI respiratory elimination in man.

INTRODUCTION

1,1,1-Trichloroethane (TRI), also known as methyl chloroform, has been used in large quantities for decades in industry as a metal degreasing solvent. Other applications include its use as a solvent in adhesives, spot removers, aerosols, and water-repellants. The toxicity of TRI is considered to be of a relatively low order of magnitude, with depression of the central nervous system (CNS) (Torkelson and Rowe, 1981; Kleinfeld and Finer, 1966; Stewart, 1968) and cardiac arrhythmias (Dornette and Jones, 1960; Reinhardt et al., 1973; Herd et al., 1984) the major effects seen after high doses in animals and humans. Hepatic and renal toxicity have been demonstrated only after very high acute doses in animals (Plaa and Larson, 1965; Klaassen and Plaa, 1966 and 1967; Gehring, 1968). Historically, human exposures to TRI have been of greatest significance in industry and other occupational settings, where exposures are primarily by inhalation. Workers are routinely exposed to TRI vapors in open or closed (i.e. recirculating) work environments. Employees may be inadvertantly exposed to very high concentrations when there has been a spill or equipment malfunction.

Studies of the pharmacokinetics of inhaled solvents such as TRI are playing an increasingly important role in toxicology. Knowledge of the uptake, disposition, and elimination of these chemicals has become necessary for health risk assessments. There is presently little data available involving such direct measurements in laboratory animals during TRI inhalation exposures. The fate of ^{14}C-TRI has been investigated following the termination of a single 6-hr inhalation exposure in rats and mice (Schumann et al., 1982a). By 72 hr post-exposure, 87-98% of the total recovered radioactivity was excreted in the expired air. Respiratory

elimination remained approximately the same after TRI inhalation exposures were repeated 5 days/week for 6 months (Schumann et al., 1982b). However, the fraction of the total inhaled dose which is eliminated during ongoing inhalation exposures has not been delineated in laboratory animals. Likewise, the subsequent rate and magnitude of uptake have not been quantified over time during the course of TRI inhalation exposures in animals. For example, it was necessary for Schumann et al. (1982a) to obtain estimates of pharmacokinetic parameters for rats from TRI exposure data in human volunteers (Monster et al., 1979), due to the lack of direct measurements of inhaled TRI in rats.

Therefore, an objective of the current investigation was to provide direct measurements of the respiratory uptake and elimination of TRI during inhalation exposures in rats. The inhaled and exhaled breath concentrations were monitored at frequent intervals both during and following TRI inhalation, as were the minute volume and respiratory rate. Blood levels of TRI were monitored concurrently, so systemic uptake and elimination could be correlated with the respiratory measurements.

MATERIALS AND METHODS

Animals. Adult, male Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Raleigh, NC). The animals were maintained on a constant light-dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. They were housed in stainless-steel cages in a ventilated animal rack. Tap water and Ralston Purina Formulab Chow were provided ad libitum. The rats were used after at least a 14-day acclimation period, at which time their body weight ranged from 325-375 g. Solvent exposures were initiated at approximately the same time each day (1000 to 1200 hr).

Test material. 1,1,1-Trichloroethane (TRI), 98.3% minimum purity, was obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ).

Animal preparation. All rats were surgically prepared with an indwelling carotid arterial cannula, which exited the animal at the back of the neck. The rats were anesthetized for the surgical procedure by an injection of 0.8 ml/kg of a mixture consisting of ketamine HCl (100 mg/ml): acepromazine maleate (10 mg/ml): xylazine HCl (20 mg/ml) in a proportion of 3:2:1 (v:v:v). The cannulated animals were maintained in a harness and pulley system that allowed relative freedom of movement in metabolism cages during a 24-hr recovery period.

Inhalation exposures. Each cannulated rat was placed into a restraining tube of the type used in nose-only inhalation exposure chambers (Battelle-Geneve, Switzerland). A face mask designed to fit the rat was held firmly in place on the animal's head by the use of elastic straps, which were secured to the restraining tube. A miniaturized one-way breathing valve (Hans Rudolph, Inc., St. Louis, MO) was attached to the face mask so that the valve entry port was directly adjacent to the nares of the test animal. This established separate and distinct airways for the inhaled and exhaled breath streams. Inhalation and exhalation sampling ports were located immediately adjacent to the breathing valve. A known concentration of the test chemical was generated within a 70-liter gas sampling bag (Calibrated Instruments, Ardsley, NY) by injecting the appropriate quantity of the solvent into the bag filled with air. Uniform dispersion of the vapor was insured by a magnetic stirring bar within the bag. The bag was then connected in series by Teflon tubing with a pneumotachograph, a three-way connector, the breathing valve, and an empty 70-l gas collection bag. The latter bag served as a reservoir to collect exhaled gas. Thereby, a closed system was maintained to prevent release of the agent into the laboratory. TRI

inhalation exposures were initiated only after stable breathing patterns were established for the cannulated animals in the system. Just before the initiation of exposure, the solvent vapor was first drawn out of the gas sampling bag by an air pump attached to the three-way connector. In this manner, the animal was assured of being exposed to a TRI concentration in the inhaled air at the very start of the exposure that was equivalent to the target concentration in the bag, without significant dilution from dead space air in the inhalation exposure system. The test animals then were subjected to inhalation exposures to the halocarbon vapors for a 2-hr period. During this exposure period and for up to 4 hr afterward, inhaled and exhaled breath samples were taken from the sampling ports at approximately the same time as blood samples from the carotid artery cannula. Both air and blood samples were then analyzed for TRI content by gas chromatography.

Respiratory measurements and calculations. The respiration of each animal was continuously monitored. The respiratory monitoring technique was conducted according to the methods previously published in solvent exposure studies by this laboratory (Dallas et al., 1983 and 1986). The airflow created by the animal's inspiration was recorded both during and following TRI inhalation exposure in terms of minute volume (volume of respiration per minute, or V_E), respiratory rate (f), and tidal volume (V_T). An average value for these parameters for each individual animal was obtained by averaging the measurements taken at 10-min intervals during the 2-hr exposure. The mean ± SE of these average values for the 500 ppm exposure group (n=6) were: V_E = 236.3 ± 22.9 ml/min; f = 135.3 ± 6.6 breaths/min; and V_T = 1.74 ± 0.18 ml. The mean ± SE of these average values for the 50 ppm exposure group (n=6) were: V_E = 252 ± 14.7 ml/min; f=129.5 ± 13.5 breaths/min; and V_T = 1.96 ± 0.1 ml.

Since the V_E and the TRI exhaled breath concentration at each sampling point were measured, subtraction of the quantity of TRI exhaled from the animal from the amount inhaled yielded an approximation of the quantity of uptake of TRI for each sampling period (cumulative uptake, or Q_{upt}).

$$Q_{upt} = (C_{inh}V_E t) - (C_{exh}V_E t) \qquad (1)$$

where C_{inh} is the inhaled concentration and V_E and C_{exh} are the minute volume and exhaled breath measurements, respectively, made at each time point, and t is the interval of time between sampling points (every 10 min for Q_{upt}). Each successive Q_{upt} is then added to the Q_{upt} value for the previous time interval in a sequential fashion, to produce a plot of cumulative uptake over the 2-hr exposure.

For determination of the cumulative elimination of TRI during inhalation exposure, the contribution of inhaled compound in the dead space volume to the concentration of chemical measured in the exhaled breath must be accounted for. In their calculation of exhaled breath concentration, Ramsey and Andersen (1984) assumed that alveolar respiration accounts for 70% of total respiration, with 30% of total respiration delegated to the inhaled air that does not participate in alveolar ventilation. By adding instrumental dead space of the breathing valve in the exposure system in the present study to this assumed dead space, a value of 50% of total respiration was assigned to both the total dead space and to alveolar ventilation. Therefore, cumulative elimination (Q_{elim}) of TRI was estimated by

$$Q_{elim} = (C_{exh}V_E t) - (C_{inh}V_D t) \qquad (2)$$

where the total dead space is $V_D = 0.5\ V_E$ and t is the time interval between sequential sampling of the exhaled breath. As for Q_{upt}, with sequential determination of Q_{elim} it is possible to monitor the cumulative elimination of TRI during inhalation exposures. The successive elimination of TRI following exposure was also calculated as $Q_{elim} = C_{exh}V_E t$.

The percent uptake (% Upt) of the total inhaled dose up to each successive time point during the inhalation exposure period by calculated as

$$\% \text{ Upt} = \frac{Q_{upt}}{C_{inh}V_A t} \qquad (3)$$

where the alveolar ventilation is $V_A = 0.5\ V_E$, and Q_{upt} values at each successive time point were employed.

The percent elimination (% Elim) of the total inhaled dose up to each successive time point during exposure was calculated as

$$\% \text{ Elim} = \frac{Q_{elim}}{C_{inh}V_A t} \qquad (4)$$

Values for % Elim following inhalation exposure employed the terminal Q_{elim} after 120 min of TRI inhalation.

The rate of chemical input into the blood, f(t), for an inhaled gas has been expressed by Veng-Pedersen (1984) as

$$f(t) = K_1\ (Cg - K_2C_B) \qquad (5)$$

where K_1 is a positive constant and is equal to the alveolar ventilation rate (V_A); K_2 is the air to blood partition coefficient; Cg is equal to C_{inh}; and

C_B is the chemical blood concentration, which is equivalent to the arterial blood concentration measured in the current study (C_{art}).

Expressing the equation in terms of currently defined variables and using the blood to air partition coefficient (N) yields

$$f(t) = V_A (C_{inh} - C_{art}) \qquad (6)$$

Integration of equation (6) from time 0 to t (the time the inhalation was terminated) gives

$$X^o_{abs} = V_A C_{inh} t - \frac{V_A (AUC)_0^t}{N} \qquad (7)$$

where X^o_{abs} is the amount of TRI absorbed into the systemic circulation, V_A is the average alveolar ventilation during the inhalation exposure, $(AUC)_0^t$ is the area-under-the-blood-concentration-time curve for the 2-hr exposure, and N is the blood:air partition coefficient for TRI in rats.

A physiological pharmacokinetic model was used to describe the disposition of TRI in the rat (Fig. 1). It was assumed that a blood flow-limited model was adequate to characterize the tissue distribution of TRI. Compartmental volumes and organ blood flows were obtained from Ramsey and Andersen (1984) and scaled to 340 g, the mean body weight of rats used in the present study. Partition coefficients for TRI were taken from Gargas et al. (1986). Total systemic clearance was obtained from a preliminary study in rats. In this study, 50 mg/kg of TRI was administered as a 2-hr constant rate iv infusion with blood samples collected for up to 9 hr after the termination of the infusion (data not shown). The total systemic clearance

was calculated as the dose divided by the total area under the TRI blood concentration-time curve. Differential mass balance equations, incorporating the parameters listed in Table 1, that described the transport of TRI in the rat were solved with the ACSL, Advanced Continuous Simulation Language, computer program (Mitchell and Gauthier, Concord, MA). The solution to the equations provided predicted TRI concentrations over time.

Analysis of TRI in air and blood. The concentration of TRI in the inhaled and exhaled air during the inhalation exposures were measured with a Tracor MT560 gas chromatograph (Tracor Instruments, Austin, TX). Analyses for the 500 ppm inhalation exposures were conducted using a flame ionization detector (FID), while the analyses for the 50 ppm inhalation exposures were conducted using an electron capture detector (ECD). In either case, air samples were procured with a gas-tight, 1-ml syringe and injected directly onto an 8-ft X 1/8-in stainless-steel column packed with 0.1% AT 1000 on GraphPak. Standards were prepared in each of four 9-liter standard bottles which have Teflon® stoppers containing needles used for taking the air samples with the syringe. Operating temperatures were: 150°C, injection port; 200°C, FID detector; 350°C, ECD detector; 110°C, isothermal column operation. When using the ECD, gas flow rates were employed of 40 ml/min for nitrogen (carrier gas), with an additional make-up gas flow rate to the detector of 30 ml/min.

TRI levels in the blood were measured by gas chromatographic headspace analysis. Blood samples were withdrawn from the arterial cannula via a stopcock by a 1-ml syringe. Depending on the anticipated blood concentration, between 25 and 200 µl of the blood was taken from the stopcock with an Eppendorf pipette and transferred to chilled headspace vials (Perkin-Elmer,

Norwalk, CT). These vials were capped immediately with PTFE lined butyl rubber septa and washers and tightly crimped. Each sample vial was then placed into the HS-6 auto-sampler unit of a SIGMA 300 gas chromatograph (Perkin-Elmer, Norwalk, CT), where it was heated to a preset temperature by a high precision thermostat device. A precise volume of the vapor was then injected automatically into the column for analysis. Standard solutions were made and assayed by diluting calculated amounts of pure TRI in toluene, transferring to vials, and analyzing as previously described. The concentration of TRI in the blood samples was then determined from a standard curve generated from blood that was spiked with these standard solutions. The column used was an 8-ft X 1/8-in stainless-steel column packed with FFAP Chromasorb W-AW (80-100 mesh). Operating temperatures were: 200° C, injection port; 300° C, ECD detector; and column oven, 85° C. The carrier gas was 5% argon-methane, at a flow rate of 40 ml/min with a make-up gas flow rate to the detector of 20 ml/min.

RESULTS

The target concentrations for the TRI inhalation exposures were 50 and 500 ppm. The actual concentration inhaled by the animals was determined by measurements of air samples taken from the airway immediately adjacent to the breathing valve. Inhaled TRI concentrations for the six rats in each group were 491.6 ± 11 ppm for the 500 ppm exposures and 51.2 ± 1.2 ppm for the 50 ppm exposures.

TRI concentrations in the blood and exhaled breath of rats both during and following inhalation exposure to TRI are shown for 50 ppm exposures in Fig. 2 and for 500 ppm exposures in Fig. 3. The exhaled breath levels during the initial min of exposure were about 50% of the inhaled concentration.

Near steady-state levels in the exhalation of TRI were rapidly achieved (within 20-45 min) at both exposure concentrations, and were directly proportional to the inhaled concentration. The exhaled breath values during 60-120 min of the exposure indicated near steady-state concentrations, being 403.5 ± 14.6 ppm for the 500 ppm exposure group and 39.4 ± 1.2 ppm (x ± SE) for the 50 ppm exposure group. Upon cessation of TRI inhalation, the concentration of TRI declined very rapidly in the expired air of both exposure groups. Substantial concentrations of TRI were found in the blood of all animals at the first sampling time (2 min). The x ± SE for the blood concentrations from 60 to 120 min, during near-steady state, were 8.12 ± 0.51 and 0.71 ± .06 µg/ml for the 500 and 50 ppm exposure groups, respectively.

The cumulative uptake of TRI during the inhalation exposures, calculated by equation (1), are shown in Fig. 4. The total cumulative uptake of TRI during the 2-hr exposure to 500 ppm was 16.67 ± 0.93 mg (x ± SE), or approximately 48 mg/kg bw. During the 2-hr exposure to 50 ppm TRI the cumulative uptake was 2.15 ± 0.2 mg (x ± SE), or 6 mg/kg bw. The quantity of TRI absorbed into the systemic circulation during the 2-hr exposure (X°_{abs}), calculated by equation (7) was 21.1 ± 7.3 mg and 2.6 ± 0.4 mg (x ± SE) for the 500 and 50 ppm exposures, respectively. The bioavailability, obtained by dividing the absorbed amounts by the administered dose times 100, was 61.6% for the 50 ppm exposure and 55.2% for the 500 ppm exposure. Percent uptake of TRI during inhalation exposure was similar for both exposure groups (Fig. 5). The very high uptake seen in the first minutes of exposure declined 30-35% during the first hour of TRI inhalation exposure. The values for both exposure groups were between approximately 48-56% throughout the second hour of exposure, (i.e. during near steady-state).

The cumulative elimination of TRI in the exhaled breath both during and following inhalation exposure is shown in Fig. 6. During TRI exposure, the cumulative elimination is dependent on TRI in the blood and on TRI eliminated from the alveolar space that was not absorbed into the blood. The magnitude of pulmonary elimination, as determined by equation (2), was proportional to the inhalation exposure concentration. By the end of the 2-hr exposure to 50 and 500 ppm TRI, 2.1 ± 0.2 and 20.8 ± 3.0 mg (x ± SE), respectively, were eliminated from the rats in the exhaled breath. Following the termination of exposure, TRI continued to be eliminated in the exhaled breath of the exposed rats. During the 2-hr post-exposure period, an additional 0.3 and 3.3 mg of TRI were eliminated from the animals in the 50 and 500 ppm exposure groups, respectively.

The percent of the inhaled dose that was eliminated during and following the 2-hr exposures to TRI are represented as plots of cumulative elimination in Fig. 7. Percent elimination of 50 ppm TRI was slightly higher than that for 500 ppm TRI inhalation during the first 90 min of exposure. By the end of the 2-hr inhalation exposures to 50 and 500 ppm TRI, 52.5 ± 4.8 and 56.3 ± 3.8% (x ± SE), respectively, of the total inhaled dose was eliminated in the exhaled breath of exposed rats. By 2 hr post-exposure, 60.3% of the total inhaled dose of TRI had been eliminated from the 50 ppm exposed rats, while 66.4% had been eliminated in the breath of the 500 ppm exposure groups.

The model predicted blood and exhaled breath TRI concentrations are shown in Figs. 2 and 3. The predicted values for the elimination of TRI in the exhaled breath were in close agreement with the measured values both during and following inhalation exposure. The concentration-time profiles of TRI in the blood were also well-described by the physiological model, with a relatively small overestimation (about 50 ng/ml) of blood levels predicted during the 50 ppm TRI inhalation.

DISCUSSION

The present study has provided a unique approach by combining direct measurements of TRI in the exhaled breath and blood simultaneously with detailed measurements of respiration. The separation of the inhaled and exhaled breath streams by use of the one-way breathing valve afforded both sampling of the exhaled breath for halocarbon during and following exposure and measurement of the air flow in the breath stream. The breathing valve has been used previously for monitoring respiration in unanesthetized animals (Mauderly et al., 1979), but pharmacokinetic measurements were not made using this system. In the previously reported pharmacokinetic studies of inhaled TRI in animals (Eben and Kimmerle, 1974; Holmberg et al., 1977; Schumann et al., 1982 a&b), direct determinations of the exhalation of the solvent by individual animals during exposures were not made, as most of these studies employed dynamic or closed exposure chambers. Emphasis on the pharmacokinetic measurements of TRI in these studies focused primarily on measurements following the termination of exposure. Also, parameters of respiration were not monitored in these experiments. Accurate determination of the total amount of chemical absorbed or eliminated by inhalation requires monitoring of respiratory parameters. In the present study, measurement of TRI uptake was accomplished by calculation from either the blood level data or the exhaled breath data in conjunction with the monitored respiratory parameters (yielding X^{o}_{abs} and Q_{upt}, respectively). It is interesting to note that these determinations of uptake from different data sets yielded similar values. The importance of quantifying the processes of uptake/elimination during inhalation is particularly apparent for TRI, a halocarbon for which respiratory elimination is so great that much of the inhaled dose is eliminated before the end of the exposure period. By the end of 50 and

500 ppm inhalation exposures for 2 hr, 52.5 and 56.3%, respectively, of the inhaled dose was eliminated in the breath.

While there has been a paucity of respiratory elimination and blood concentration data during TRI inhalation in laboratory animals, there have been some studies involving these determinations in humans. Since TRI itself has a relatively low degree of acute toxicity and is not metabolized to a significant extent in the body to toxic metabolites, it has been selected as an appropriate chemical for evaluation of the uptake and elimination of volatile aliphatic halocarbons in humans. The major route of excretion of inhaled TRI in humans was found to be respiratory elimination of the parent compound (Stewart et al., 1969; Morgan et al., 1972; Caperos et al., 1982; Nolan et al., 1984). Other TRI inhalation studies in humans have focused on the relatively limited urinary excretion of TRI metabolites (Seki et al., 1975; Ikeda and Ohtsuju, 1972) and the effect of exercise on the uptake of TRI (Astrand et al., 1973; Monster et al., 1979). Six-hr inhalation exposures to TRI have been conducted with male human volunteers, and direct measurements of TRI in the exhaled breath and blood made during and following the exposures (Nolan et al., 1984). The exhaled breath levels after 1.5 hr of exposure to 35 and 350 ppm of TRI were 0.14 and 1.28 µg/ml, respectively. Assuming a linear scale-up to a 50 and 500 ppm exposure (0.2 and 1.83 µg/ml, respectively), these exhaled breath levels in humans are very similar to exhaled levels measured after 1.5 hr of exposure in the present study in rats (0.21 and 2.16 µg/ml, respectively). In both the human study and the rat study reported here, TRI elimination in the breath was proportional to the exposure concentration.

While TRI exhaled breath levels in rats in this study were comparable to those measured previously in humans, blood levels of TRI were not equivalent in rats and man. After 1.5 hr of exposure to 35 or 350 ppm TRI, human

volunteers had mean blood levels of 0.14 and 1.62 µg/ml, respectively (Nolan et al., 1984). After normalizing for differences in exposure concentrations between the studies, mean blood levels in rats in the present investigation were approximately 3.6 times higher than levels seen in humans. Schumann et al. (1982a) reported blood levels of ^{14}C-TRI in rats similar to TRI levels measured in rats in the present study (when normalized for exposure concentration). In a comparison with the data of Schumann et al. (1982a), Nolan et al. (1984) pointed out that the blood levels in mice and rats inhaling TRI were 17.3 and 3.5 times higher than those they measured in human volunteers. Nolan et al. (1984) concluded that the rat was a better model than the mouse in evaluating human health effects of TRI, on the basis of the blood level data and interspecies comparisons of TRI absorption and metabolism.

It is unclear why exhaled breath levels of TRI in rats in the present investigation were so similar to those reported in humans (Nolan et al., 1984), while blood levels in the two species were significantly different. While this comparison is based on an equivalent inhaled concentration in the two species, consideration of the actual inhaled dose that was received must incorporate the wide variation in volume of respiration and body weight between rats and man. Assuming a 4.2 l/min alveolar ventilation and 70 kg body weight for man (Ganong, 1979), the rats in the present study received an inhaled dose approximately six times greater than did the humans in the study by Nolan et al. (1984). This is based, of course, on the premise that the % uptake of TRI in rats and man is the same, as has been assumed in previous TRI inhalation studies (Schumann et al., 1982a). This phenomenon may provide one explanation for the higher blood levels observed in rats. Nevertheless, there was equivalent respiratory elimination of TRI in the two species, despite an apparently higher inhaled dose received by rats relative to man.

There is a growing interest in developing physiological pharmacokinetic models for prediction of disposition of toxic chemicals in humans. Confidence in the use of scaled animal-based models to predict the kinetics of chemicals in humans can be gained only if the animal model can accurately predict chemical concentrations measured in the animal. The model utilized in the present investigation predicted blood and exhaled breath concentrations of TRI reasonably well, as determined by comparison with direct measurements. However, the model will require further evaluation before it can serve as a predictor for TRI tissue concentrations. To this end, measurement of rat TRI tissue concentrations will be necessary for additional refinements and verification of the present model. In view of the finding that TRI exhaled breath concentrations in rats and humans were very similar, while blood levels were not, it will be of particular interest to determine if such an appropriately validated model can predict both TRI exhaled breath and blood levels in humans with similar accuracy. As the reason for this observed difference between the species is not known, such simulations using a physiological basis for prediction may provide additional insight into this phenomenon.

REFERENCES

ASTRAND, I., KILBOM, A., WAHLBERG, I., AND OVRUM, P. (1973). Methyl-chloroform exposure. I. Concentration in alveolar air and blood at rest and during exercise. Work Environ Health 9, 69-81.

CAPEROS, J. R., DROZ, P. O., HAKE, C. L., HUMBERT, B. E., AND JACOT-GUILLARMOD, A. (1982). 1,1,1-Trichloroethane exposure, biological monitoring by breath and urine analysis. Int. Arch. Occup. Environ. Health 49, 293-303.

DALLAS, C. E., WEIR, F. W., FELDMAN, S., PUTCHA, L., AND BRUCKNER, J. V. (1983). "The uptake and disposition of 1,1-dichloroethylene in rats during inhalation exposure". Toxicol. Appl. Pharmacol. 68, 140-151.

DALLAS, C. E., BRUCKNER, J. V., MAEDGEN, J. L., AND WEIR, F. W. (1986). "A method for direct measurement of systemic uptake and elimination of volatile organics in small animals." J. Pharmacol. Methods 16, 239-250.

DORNETTE, W. H. L., AND JONES, J. P. (1960). Clinical experiences with 1,1,1-trichloroethane. A preliminary report of 50 anesthetic administrations. Anest. Analg. 39, 249-253.

EBEN, A., AND KIMMERLE, G. (1974). Metabolism, excretion and toxicology of methyl chloroform in acute and subacute exposed rats. Arch. Toxikol. 31, 233-242.

GANONG, W. F. (1979). Review of Medical Physiology, p. 500. Lange Medical Publications, Los Altos, California.

GARGAS, M. L., ANDERSEN, M. E., AND CLEWELL, H. J., III (1986). A physiologically-based simulation approach for determining metabolic constants from gas uptake data. Toxicol. Appl. Pharmacol. 86, 341-352.

GEHRING, P. J.(1968). Hepatoxic potency of various chlorinated hydrocarbon vapors relative to their narcotic and lethal potencies in mice. Toxicol. Appl. Pharmacol. 13, 287-293.

HERD, P. A., LIPSKY, M., AND MARTIN, H. F. (1974). Cardiovascular effects of 1,1,1-trichloroethane. Arch. Environ. Health 28, 227-233.

HOLMBERG, B., JAKOBSON, I., AND SIGVARDSSON, K. (1977). A study on the distribution of methyl chloroform and n-octane in the mouse during and after inhalation. Scand. J. Work Environ. Health 3, 43-52.

IKEDA, M., AND OHTSUJI, H.(1972). A comparative study of the excretion of Fujiwara reaction-positive substances in urine of humans and rodents given trichloro- or tetrachloro-derivatives of ethane and ethylene. Brit. J. Ind. Med. 29, 99-104.

KLAASSEN, C. D., AND PLAA, G. L. (1966). Relative effects of various chlorinated hydrocarbons on liver and kidney function in mice. Toxicol. Appl. Pharmacol. 9, 139-151.

KLAASSEN, C. D., AND PLAA, G. L. (1966). Relative effects of various chlorinated hydrocarbons on liver and kidney function in dogs. Toxicol. Appl. Pharmacol. 10, 119-131.

KLEINFELD, M., AND FEINER, B. (1966). Health hazards associated with work in confined spaces. J. Occup. Med. 8, 358-364.

MAUDERLY, J. L., TESAREK, J. E., SIFFORD, L. J., AND SIFFORD, L. J. (1979). Respiratory measurements of unsedated small laboratory mammals using nonrebreathing valves. Lab Anim. Sci. 29, 323-329.

MONSTER, A. C., BOERSMA, G., AND STEENWEG, H. (1979). Kinetics of 1,1,1-trichloroethane in volunteers: Influence of exposure concentration and work load. Int. Arch. Occup. Environ. Health 42, 293-302.

MORGAN, A., BLACK, A., AND BELCHER, D. R. (1972). Studies on the absorption of halogenated hydrocarbons and their excretion in breath using 38Cl tracer techniques. Ann. Occup. Hyg. 15, 273-282.

NOLAN, R. J., FRESHOUR, N. L., RICK, D. L., McCARTY, L. P., AND SAUNDERS, J. H. (1984). Kinetics and metabolism of inhaled methyl chloroform (1,1,1-trichloroethane) in male volunteers. Fund. Appl. Toxicol. 4, 654-662.

PLAA, G. L., AND LARSON, R. E. (1965). Relative nephrotoxic properties of chlorinated methane, ethane, and ethylene derivatives in mice. Toxicol. Appl. Pharmacol. 7, 37-44.

RAMSEY, J. C., AND ANDERSEN, M. E. (1984). A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. Toxicol. Appl. Pharmacol. 73, 159-175.

REINHARDT, C. F., MULLIN, L. S. AND MAXFILED, M. E. (1973). Epinephrine-induced cardiac arrhythmic potential of some common industrial solvents. J. Occup. Med. 15, 953-955.

SCHUMANN, A. M., FOX, T. R., AND WATANABE, P. G. (1982a). ^{14}C-Methyl chloroform (1,1,1-trichloroethane): Pharmacokinetics in rats and mice following inhalation exposure. Toxicol. Appl. Pharmacol. 62, 390-401.

SCHUMANN, A. M., FOX, T. R., AND WATANABE, P. G. (1982b). A comparison of the fate of inhaled methyl chloroform (1,1,1-trichloroethane) following single or repeated exposure in rats and mice. Fund. Appl. Toxicol. 2, 27-32.

SEKI, Y., URASHIMA, Y., AIKAWA, H., MATSUMURA, H., ICHIKAWA, Y., HIRATSUKA, F., YOSHIOKA, Y., SHIMBO, S., AND IKEDA, M. (1975). Trichloro-compounds in the urine of humans exposed to methyl chloroform at subthreshold levels. Int. Arch. Arbeitsmed. 34, 39-49.

STEWART, R. D. (1968). The toxicology of 1,1,1-trichloroethane. Ann. Occup. Hyg. 11, 71-79.

STEWART, R. D., GAY, H. H., SCHAFFER, A. W., ERLEY, D. S., AND ROWE, V. K. (1969). Experimental human exposure to methyl chloroform vapor. Arch. Environ. Health 19, 467-472.

TORKELSON, T. R., AND ROWE, V. K. (1981). Halogenated aliphatic hydrocarbons containing chlorine, bromine and iodine. In Patty's Industrial Hygiene and Toxicology (G. D. Clayton and E. Clayton, eds.), 3rd ed., pp. 3502-3510. Wiley, New York.

VENG-PEDERSEN, P. (1984). Pulmonary absorption and excretion of compounds in the gas phase: Theoretical pharmacokinetic and toxicokinetic analysis. J. Pharm. Sci. 73, 1136-1141.

Figure Legends

1. Diagram of the physiological pharmacokinetic model used to simulate the uptake and elimination of inhaled TRI. The symbols and parameters used to describe the model are in Table I and in the equations given in the Materials and Methods section.
2. Observed (•) and model-predicted (-) TRI concentrations in the blood (top graph) and exhaled breath (bottom graph) of rats during and following a 2-hr 50 ppm inhalation exposure. Each observed value represents the mean for 6 rats.
3. Observed (•) and model-predicted (-) TRI concentrations in the blood (top graph) and exhaled breath (bottom graph) of rats during and following a 2 hr 500 ppm inhalation exposure.
4. Cumulative uptake of TRI during inhalation exposures. Rats inhaled 50 or 500 ppm TRI for 2 hr. The quantity of inhaled TRI retained during successive 10-min intervals was calculated on the basis of the measured minute volume and difference between inhaled and exhaled TRI concentrations. Each point represents the mean ± SE for 6 rats.
5. Percent uptake of TRI during inhalation exposures. Rats inhaled 50 or 500 ppm TRI for 2 hr. Each point represents the mean ± SE for 6 rats. The percent uptake of the inhaled dose over time was determined after 1, 3, 5, 10, 15, and 20 min and at 10-min intervals thereafter.
6. Cumulative elimination of TRI during and following inhalation exposures. Rats inhaled 50 or 500 ppm TRI for 2 hr. The quantity of inhaled TRI eliminated in the breath over time was calculated using direct measurements of the minute volume and TRI concentrations in the inhaled

and exhaled breath. The contribution of inhaled TRI from instrumental and anatomic dead space to the quantity exhaled was deleted. Each point is the mean ± SE for 6 rats. Cumulative elimination was determined for successive 10-min intervals during the 2-hr exposure, and for successive 15-min intervals post-exposure.

7. Percent elimination of TRI during and following inhalation exposures. Rats inhaled 50 or 500 ppm TRI for 2 hr. Each point represents the mean ± SE for 6 rats. The percent of the inhaled dose that was eliminated over time was determined after 3, 5, and 10 min and at 10-min intervals thereafter during exposure, and at 15-min intervals post-exposure.

FOOTNOTES

[1] Research sponsored by U.S. EPA Cooperative Agreement CR 812267 and the Air Force Office of Scientific Research, Air Force Systems Command, USAF, under grant number AFOSR 87-0248. The US Government is authorized to reporduce and distribute reprints for Governmental purposes notwithstanding any copyright notation thereon. This manuscript is submitted for publication with the understanding tha the US Government is authorized to reproduce and distribute reprints for Governmental purposes.

[2] Presented at the 26th Annual Meeting of the Society of Toxicology, Washington, DC, February, 1987.

[3] To whom correspondence should be addressed.

ACKNOWLEDGEMENT

The authors are grateful to Ms. Linda Quigley, Miss Elizabeth Lehman and Mrs. Judy Bates for their expertise in preparation of this manuscript.

INDEX TERMS

1,1,1-Trichloroethane

Methyl Chloroform

Physiologically-based Pharmacokinetic Model

Respiratory Elimination

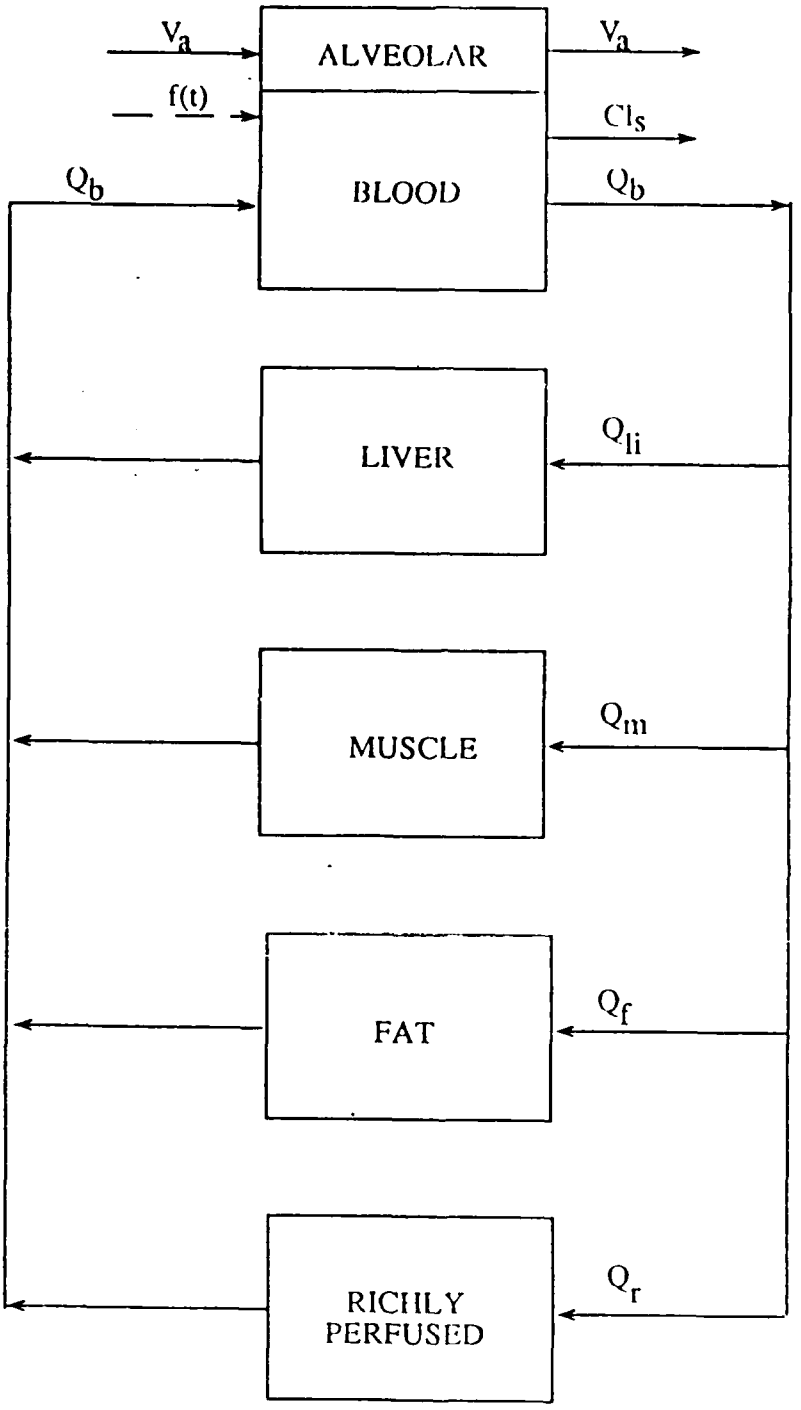
Pharmacokinetics

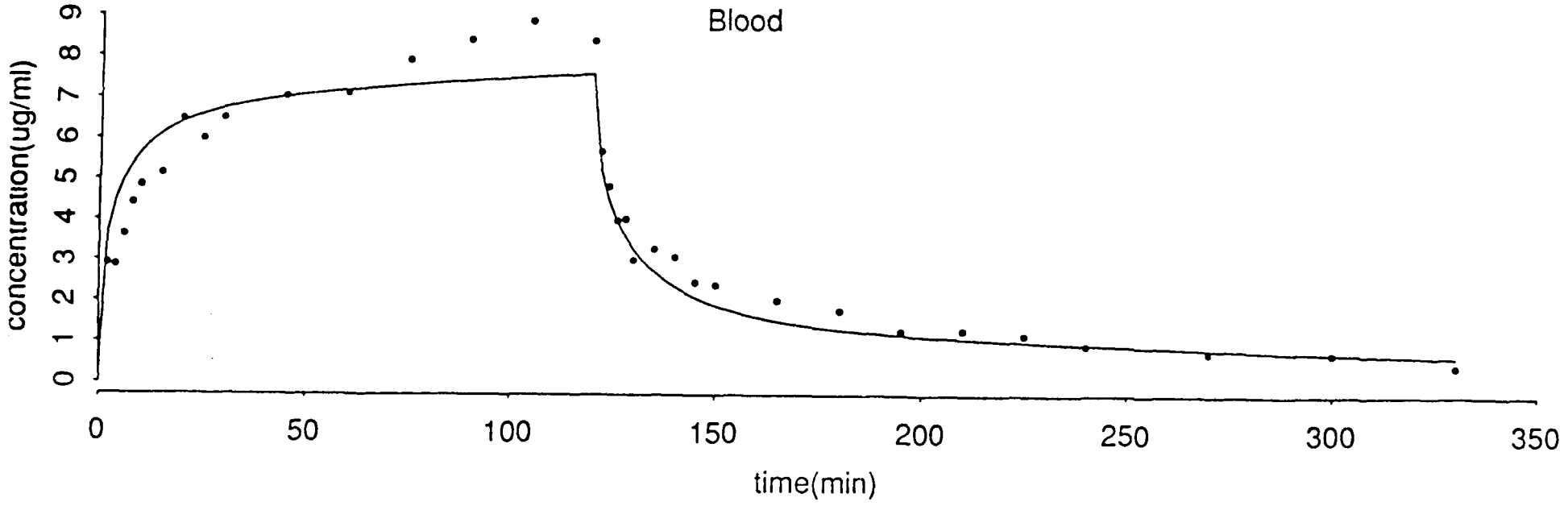
Inhalation Exposure

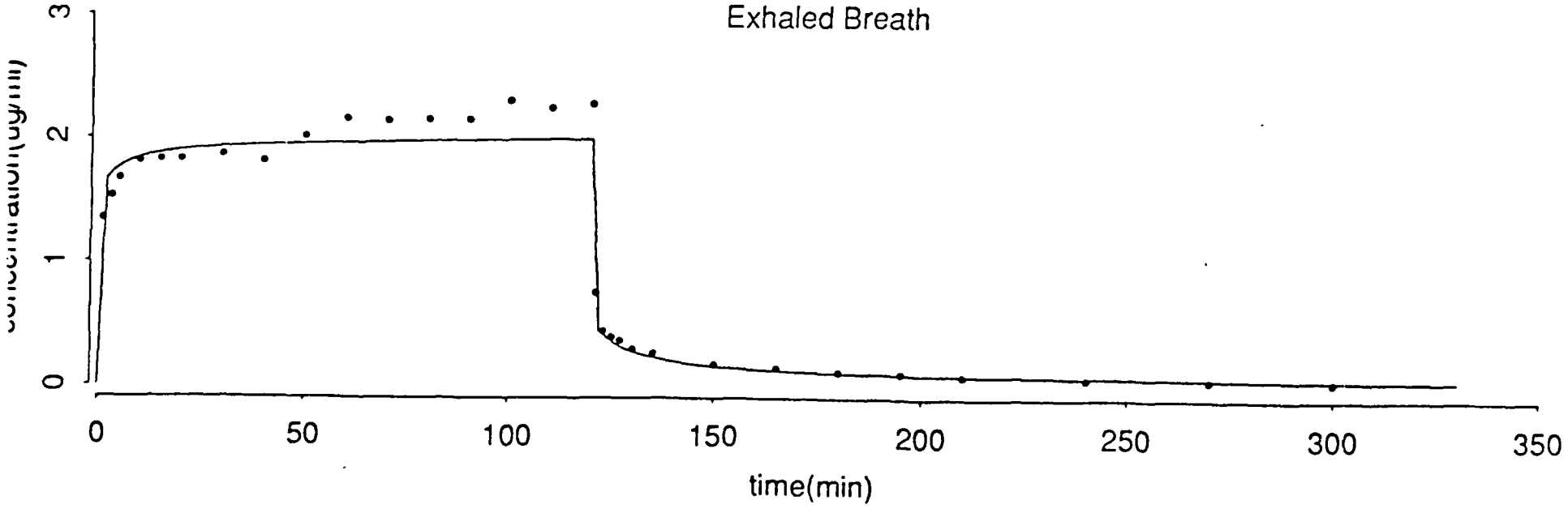
Interspecies Extrapolations

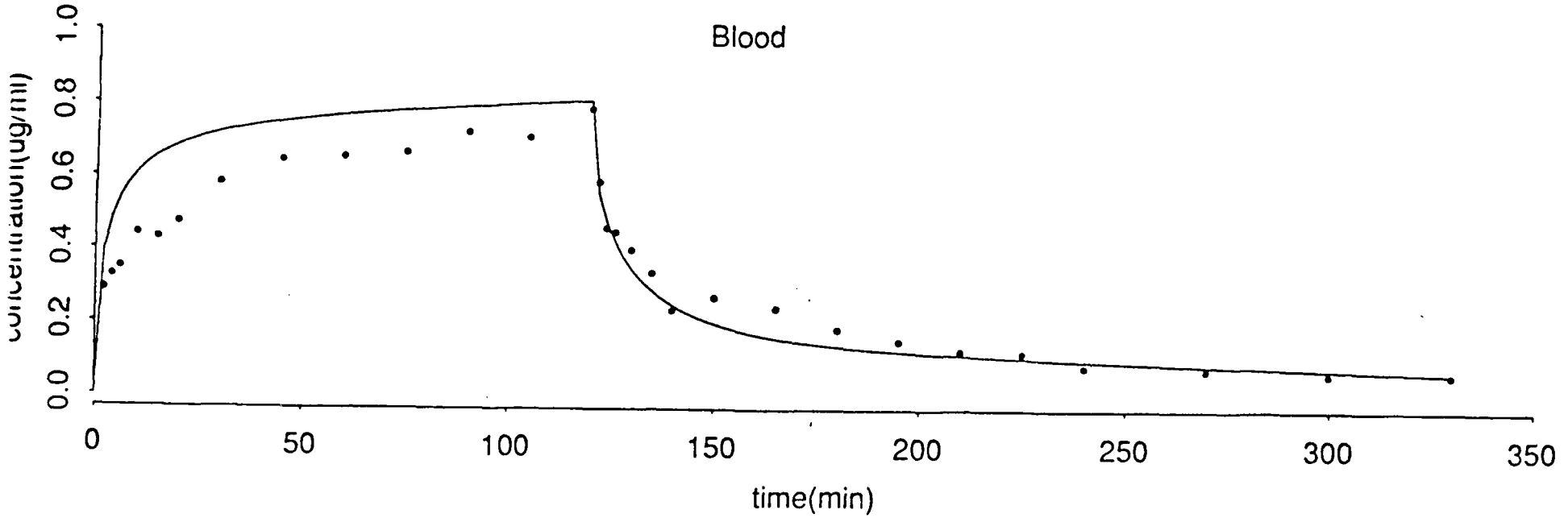
Table 1. Parameters for the Physiological Pharmacokinetic Model of TRI in the Rat (340 g)

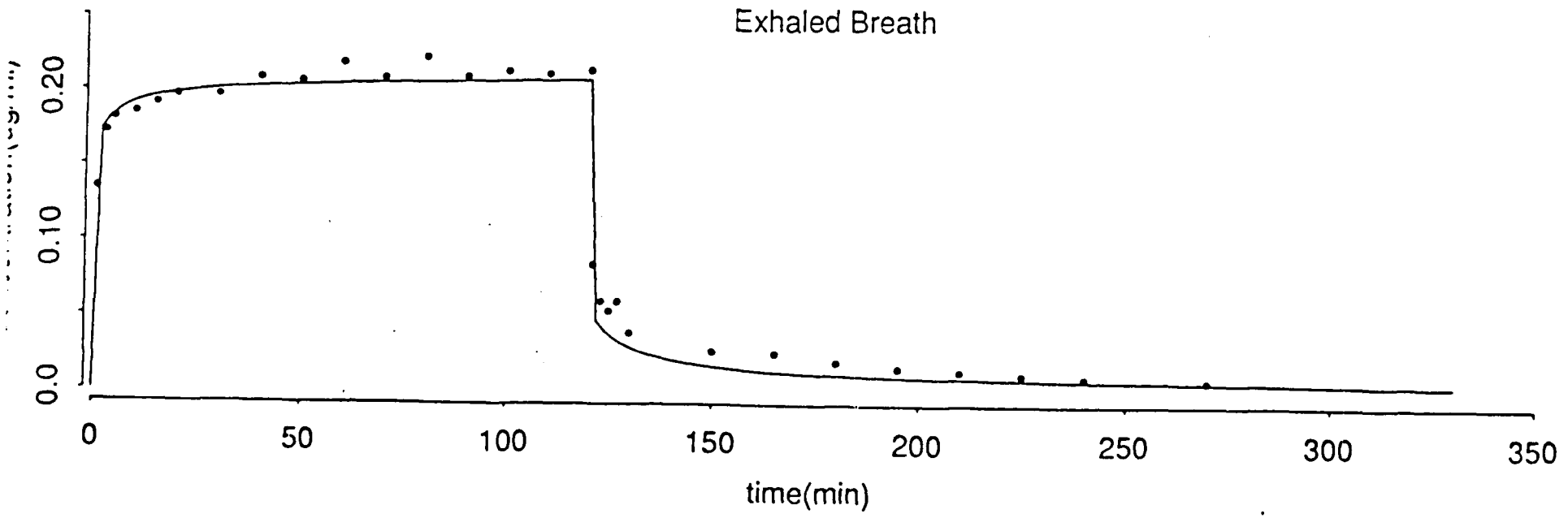
Parameter	Value
Alveolar Ventilation Rate (ml/min), V_a	126 (50 ppm exposure)
	118 (500 ppm exposure)
Inhaled Gas Concentration(µg/ml)	0.279 (50 ppm exposure)
	2.70 (500 ppm exposure)
Total Systemic Clearance (ml/min), Cl_s	17 ml/min
Blood Flows (ml/min)	
Cardiac output, Q_b	106.4
Fat, Q_f	9.4
Liver, Q_{li}	39.8
Muscle, Q_m	12.8
Richly Perfused, Q_r	44.4
Tissue Volumes (ml)	
Blood	25.4
Fat	30.5
Liver	13.6
Muscle	248.0
Richly Perfused	17.0
Partition Coefficients	17.0
Blood:Air	5.8
Fat:Blood	47.7
Liver:Blood	1.49
Muscle:Blood	0.55
Richly Perfused: Blood	5.7

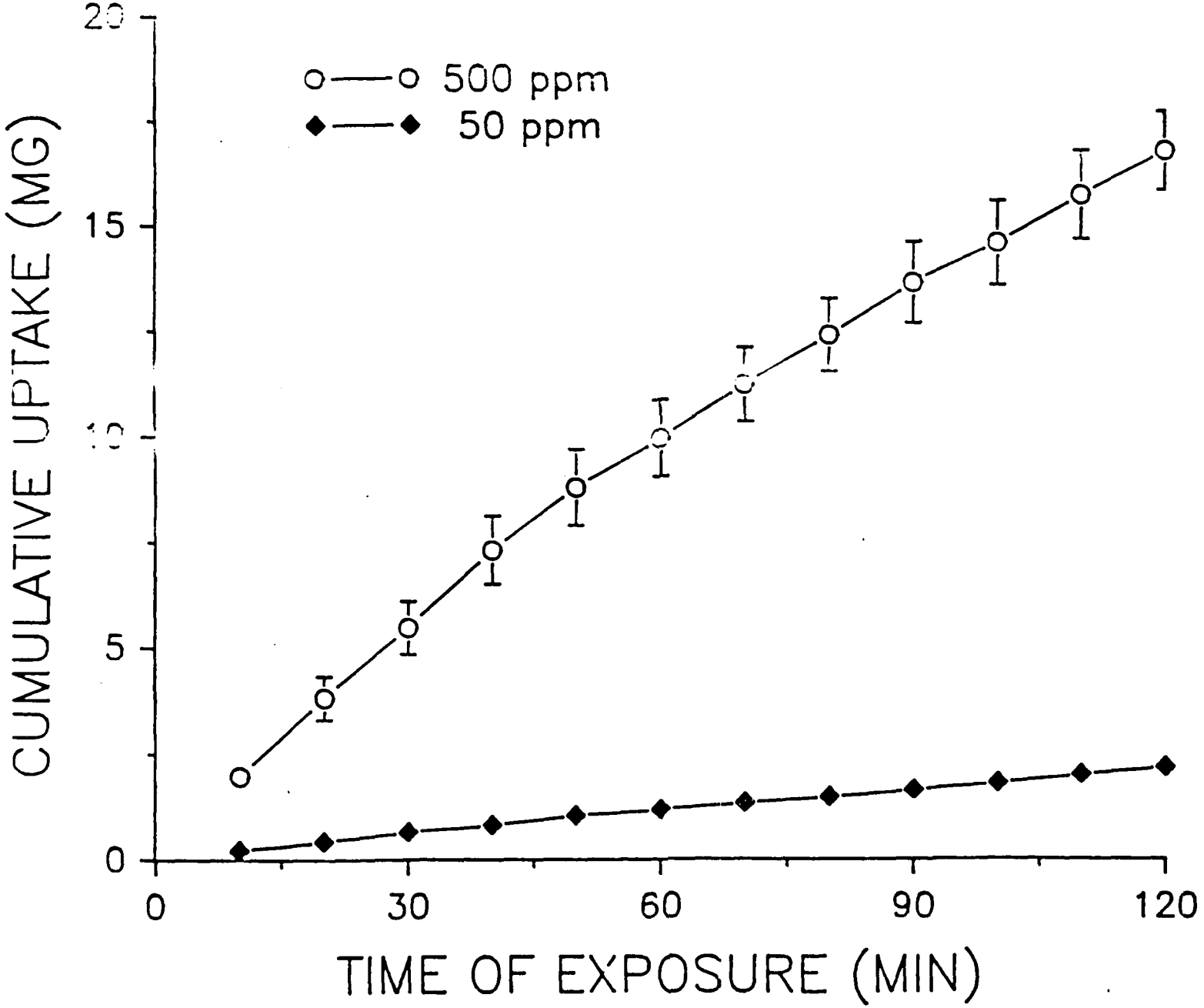

V_a
ALVEOLAR
V_a
f(t)
Cl_s
Q_b
BLOOD
Q_b
LIVER
Q_{li}
MUSCLE
Q_m
FAT
Q_f
RICHLY PERFUSED
Q_r

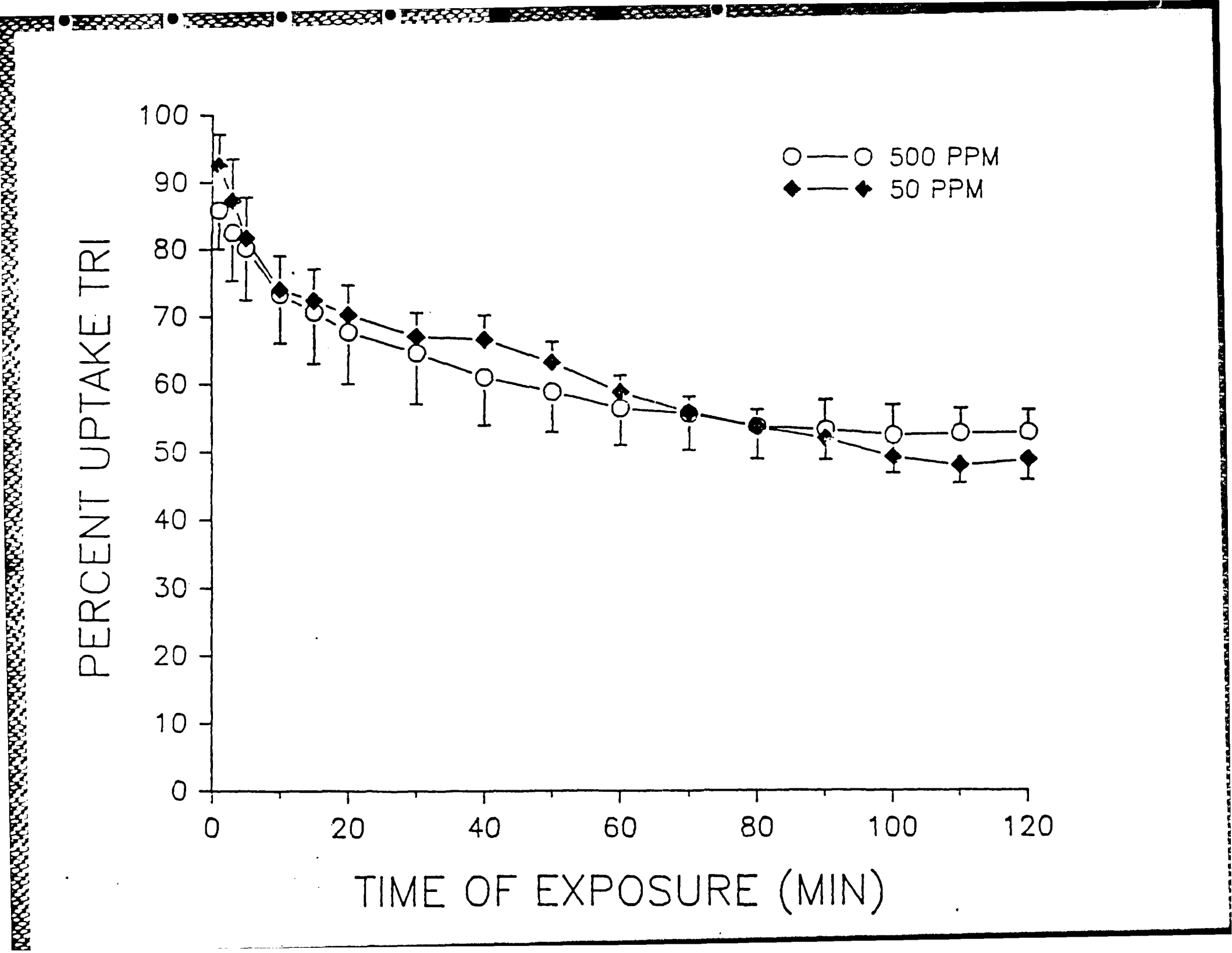

Blood
concentration(ug/ml)
0 1 2 3 4 5 6 7 8 9
0
50
100
150
200
250
300
350
time(min)

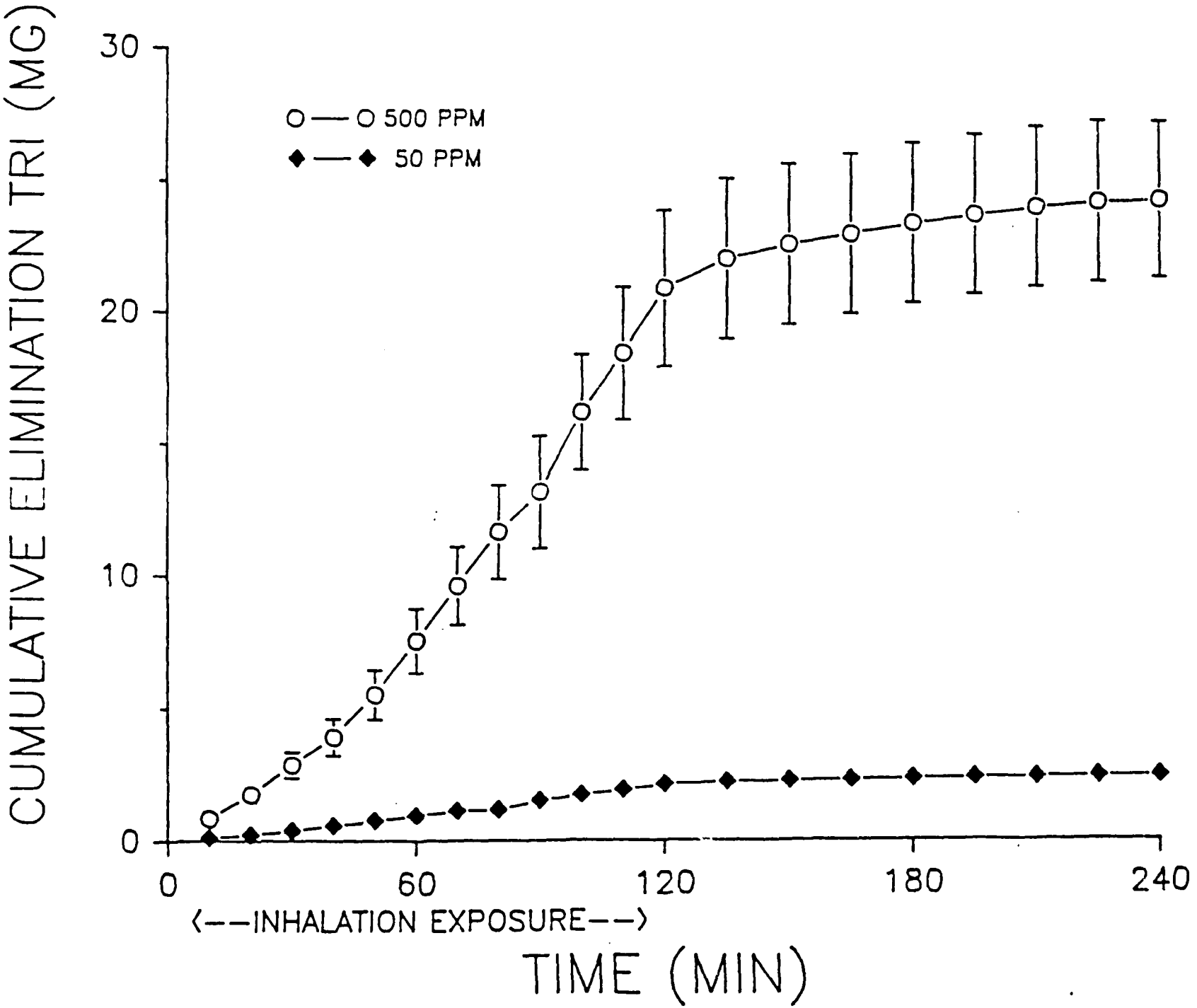

Exhaled Breath
0
1
2
3
0
50
100
150
200
250
300
350
time(min)

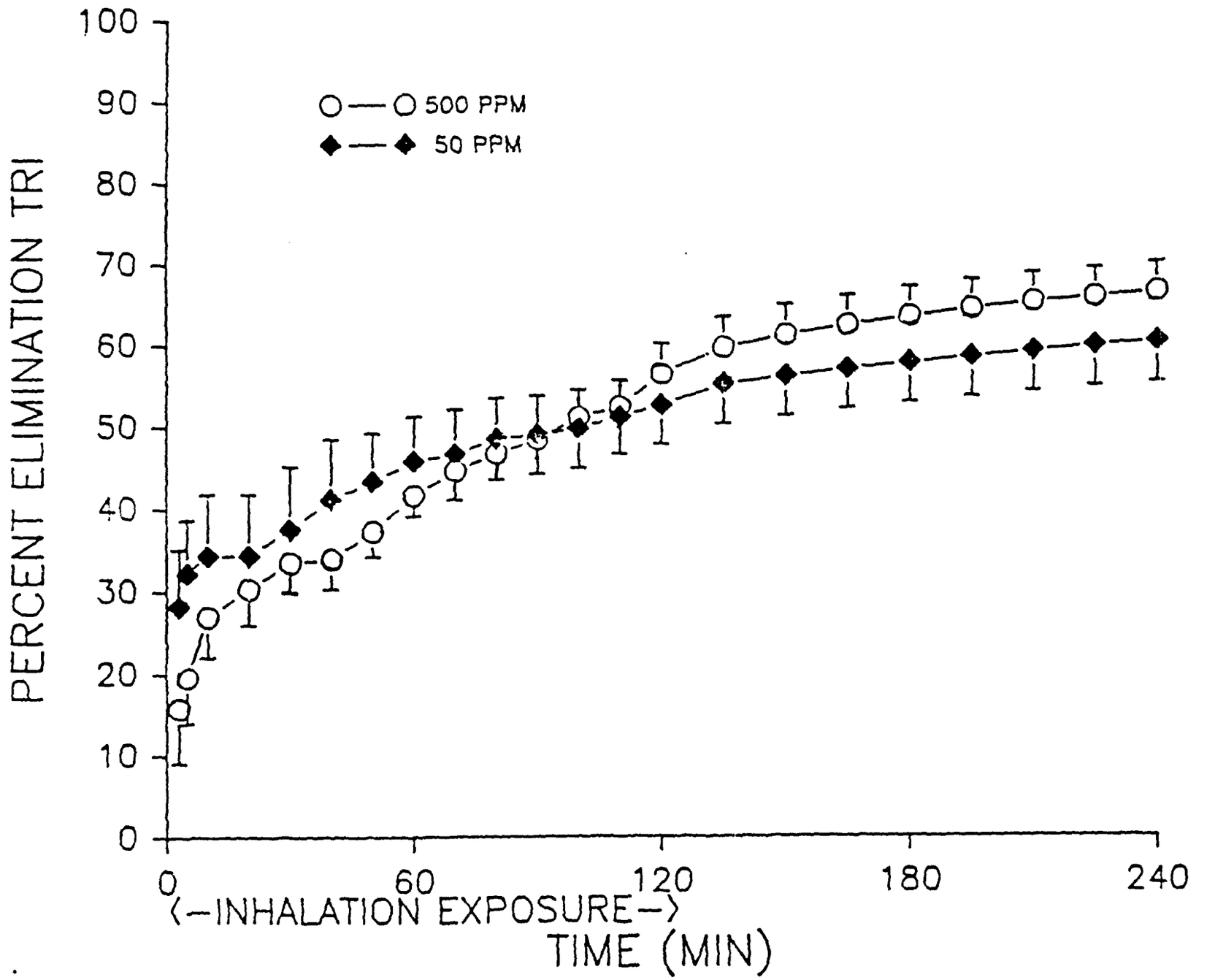

Blood
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200
250
300
350
time(min)


Exhaled Breath
0.0
0.10
0.20
0
50
100
150
200
250
300
350
time(min)


20
15
5
0
o—o 500 ppm
◆—◆ 50 ppm
CUMULATIVE UPTAKE (MG)
0
30
60
90
120
TIME OF EXPOSURE (MIN)


100
90
80
70
60
50
40
30
20
10
0
PERCENT UPTAKE TRI
500 PPM
50 PPM
0
20
40
60
80
100
120
TIME OF EXPOSURE (MIN)


CUMULATIVE ELIMINATION TRI (MG)
30
20
10
0
O — O 500 PPM
◆ — ◆ 50 PPM
0
60
120
180
240
⟨--INHALATION EXPOSURE--⟩
TIME (MIN)


PERCENT ELIMINATION TRI
100
90
80
70
60
50
40
30
20
10
0
O — O 500 PPM
◆ — ◆ 50 PPM
0
60
120
180
240
<—INHALATION EXPOSURE—>
TIME (MIN)

APPENDIX B

MANUSCRIPT NOW BEING SUBMITTED TO THE JOURNAL

FUNDAMENTAL AND APPLIED TOXICOLOGY

Dallas, C. E., Ramanathan, R., Muralidhara, S., Gallo, J. M. and Bruckner, J. V. "Direct Measurement of trichloroethylene (TCE) in the blood and exhaled breath of rats during and following inhalation exposures". To be submitted to Fundamental and Applied Toxicology (1988).

Direct Measurements of Trichloroethylene (TCE) in the Blood and Exhaled Breath of Rats During and Following Inhalation Exposures[1,2]

CHAM E. DALLAS[3], RAGHUPATHY RAMANATHAN, SRINIVASA MURALIDHARA, JAMES M. GALLO*, AND JAMES V. BRUCKNER

Department of Pharmacology and Toxicology
*Department of Pharmaceutics
College of Pharmacy
University of Georgia
Athens, GA 30602

Abbreviated title: Kinetics of Inhaled Trichloroethylene

Please send all correspondence to:

Dr. Cham E. Dallas
Department of Pharmacology and Toxicology
College of Pharmacy
University of Georgia
Athens, GA 30602

Direct Measurements of Trichloroethylene (TCE) in the Blood and Exhaled Breath of Rats During and Following Inhalation Exposure. Dallas, C. E., Ramanathan, R., Muralidhara, S., Gallo, J. M., and Bruckner, J. V. (1988). Fund. Appl. Toxicol. 0,000-000. The pharmacokinetics of trichloroethylene (TCE) was studied in male Sprague-Dawley rats in order to characterize and quantify TCE uptake, respiratory elimination and metabolism by direct measurements of the inhaled compound. Fifty or 500 ppm TCE was inhaled for 2 hr through a one-way breathing valve by unanesthetized rats of 325-375 g. Repetitive samples of the separate inhaled and exhaled breath streams, as well as arterial blood, were collected concurrently both during and following TCE inhalation and analyzed for TCE by gas chromatography. Respiratory rates and volumes were continuously monitored during and following exposure, and were used in conjunction with the pharmacokinetic data to characterize profiles of uptake and elimination. TCE exhaled breath levels increased rapidly after the initiation of exposure to near steady-state within approximately 20-30 min and were then directly proportional to the exposure concentration. Uptake of TCE in the blood was also rapid, but blood levels continued to increase progressively over the course of the inhalation exposure at both dose levels. Arterial TCE concentrations were not proportional to the inhalation concentration, with levels for the 500 ppm group from 25-30 times greater than in 50 ppm-exposed rats during the second hour of the exposure. Percent uptake was nearly complete at the initiation of inhalation exposures and decreased rapidly thereafter until equilibrium was established by 1 hr, after which % uptake of the inhaled dose was approximately 69-72% for both exposure groups. Total cumulative uptake of 50 and 500 ppm TRI over the 2-hr

inhalation exposures was determined to be 8.4 and 73.3 mg/kg bw, respectively. The direct measurements of TCE in the blood and exhaled breath were utilized in the validation of a physiological pharmacokinetic (PBPK) model for the prediction of the pharmacokinetics of inhaled TCE. The PBPK model was characterized as blood flow-limited with TCE eliminated unchanged in the exhaled breath and by saturable liver metabolism. Predicted TCE blood and exhaled breath concentrations agreed well with observed values at both the 50 and 500 ppm exposure. Exhaled breath levels of TCE in rats were similar in magnitude to values previously published for TCE inhalation exposures in humans.

Trichloroethylene (1,1,2-trichloroethylene, TCE) is a volatile industrial solvent which has been widely used in industrial degreasing of metals, dry cleaning, and other commercial applications. Of the current U.S. production of about 130,000 metric tons per year, approximately 80 to 95 per cent evaporates into the air (U.S. EPA, 1985). It has been estimated that of the 3.5 million persons occupationally exposed to TCE, full-time exposures exist for at least 100,000 workers and that two-thirds of these are in work environments where there are not adequate control measures (NIOSH, 1978). Occupational overexposures to TCE vapors have resulted in reports of central nervous system depression in workers, including loss of equilibrium, lack of coordination, and unconsciousness (Williams 1959; Longley and Jones, 1963). While lethal respiratory depression and cardiac arrhythmias have occurred with very severe TCE inhalation, most occupational exposures are believed to have resulted in relatively few manifestations of toxicity in humans (Defalque, 1965; NIOSH, 1973; EPA, 1985). Carcinogenicity from the repeated daily administration of high oral doses of TCE has been demonstrated in B6C3F1 mice, but not in several other rodent species tested (NCI, 1976; NTP, 1983).

There is presently a paucity of data involving direct measurements of TCE in the blood and exhaled breath of laboratory animals during inhalation exposures. In an examination of species differences in TCE inhalation pharmacokinetics, rats and mice received 10 and 600 ppm inhalation exposures to ^{14}C-TCE (Stott et al., 1982). In evaluating the proportion of the net body uptake that was metabolized, there apparently was saturation of TCE metabolism in the rat but not in the mouse at the high dose. In this study, no measurements of TCE in the blood were

made, and TCE in the exhaled breath was determined only at 50 hr post-exposure. In other studies of the metabolism of inhaled TCE, indirect measurements of TCE uptake and metabolism have been made by measuring the rate of depletion of TCE from the atmosphere of a closed system containing laboratory animals (Filser and Bolt, 1979; Andersen et al., 1980). Direct measurements of TCE in the blood were not conducted in these studies, either. In a recent study of TCE metabolism in rats relative to its induction by ethanol or phenobarbital (Nakajima et al., 1988), blood levels of unchanged TCE were determined following the termination of inhalation exposure. Otherwise, the existing TCE blood concentration-time data that is available for TCE exposures in rats presently involves oral or intravenous administration of the compound (Withey and Collins, 1980; D'Souza et al., 1985). Therefore, one of the objectives of the present investigation was to define the systemic uptake and disposition of TCE in the blood during and following inhalation exposure in rats.

While measurements of the body burden of ^{14}C-TCE have been conducted following the termination of inhalation exposure in rodents, including rats (Stott et al., 1982), the rate and magnitude of the uptake of TCE have not been quantified over time during the course of TCE inhalation exposures in rodents. Also, the amount of the total inhaled dose which is eliminated during ongoing TCE inhalation exposure has not been delineated in laboratory animals. To this end, direct measurements of the inhaled and exhaled breath concentrations were monitored during and following TCE inhalation, concurrently with respiratory monitoring of the minute volume and respiratory rate.

Direct measurements of TCE in the blood and the exhaled breath were then compared to predicted values obtained from a physiologically-based pharmacokinetic (PBPK) model.

MATERIALS AND METHODS

Animals. Adult, male Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Raleigh, NC). The animals were maintained on a constant light-dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. They were housed in stainless-steel cages in a ventilated animal rack. Tap water and Ralston Purina Formulab Chow were provided ad libitum. The rats were used after at least a 14-day acclimation period, at which time their body weight ranged from 325-375 g. Solvent exposures were initiated at approximately the same time each day (1000 to 1200 hr).

Test material. Trichloroethylene (TCE), of > 99.99% purity, was obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ).

Animal preparation. All rats were surgically prepared with an indwelling carotid arterial cannula, which exited the animal at the back of the neck. The rats were anesthetized for the surgical procedure by an injection of 0.8 ml/kg of a mixture consisting of ketamine HCl (100 mg/ml): acepromazine maleate (10 mg/ml): xylazine HCl (20 mg/ml) in a proportion of 3:2:1 (v:v:v). The cannulated animals were maintained in a harness and pulley system that allowed relative freedom of movement in metabolism cages during a 24-hr recovery period.

Inhalation exposures. A known concentration of the test chemical was generated within a 70-liter gas sampling bag (Calibrated

Instruments, Ardsley, NY) by injecting the appropriate quantity of the solvent into the bag filled with air. Uniform dispersion of the vapor was insured by a magnetic stirring bar within the bag. The bag was then connected in series by Teflon tubing with a pneumotachograph, a miniaturized one-way breathing valve (Hans Rudolph, Inc. St. Louis, MO), and an empty 70-l gas collection bag. The latter bag served as a reservoir to collect exhaled gas. The breathing valve was attached to a face mask designed to fit the rat so that the valve entry port was directly adjacent to the nares of the test animal, thus establishing separate and distinct airways for the inhaled and exhaled breath streams. Inhalation and exhalation sampling ports were located immediately adjacent to the breathing valve. A cannulated rat was placed into a restraining tube of the type used in nose-only inhalation exposure chambers (Battelle-Geneve, Switzerland) and the face mask held firmly in place on the animal's head by the use of elastic straps, which were secured to the restraining tube. TCE inhalation exposures were initiated only after stable breathing patterns were established for the cannulated animals in the system. The test animals then inhaled TCE vapors for a 2-hr period. During this exposure period and for up to 4 hr afterward, inhaled and exhaled breath samples were taken from the sampling ports at approximately the same time as blood samples from the carotid artery cannula. Both air and blood samples were then analyzed for TCE content by gas chromatography.

Respiratory measurements and calculations. The respiration of each animal was continuously monitored according to the methods previously published in solvent exposure studies by this laboratory (Dallas et al., 1983 and 1986). The airflow created by the animal's inspiration was

recorded both during and following TCE inhalation exposure in terms of minute volume (volume of respiration per minute, or V_E), respiratory rate (f), and tidal volume (V_T). An average value for these parameters for each individual animal was obtained by averaging the measurements taken at 10-min intervals during the 2-hr exposure. The mean ± SE of these average values for the 500 ppm exposure group (n=6) were: V_E = 218.0 ± 20.21 ml/min; f = 128.4 ± 7.1 breaths/min; and V_T = 1.71 ± 0.15 ml. The mean ± SE of these average values for the 50 ppm exposure group (n=6) were: V_E = 268.9 ± 15.5 ml/min; f=132.0 ± 7.3 breaths/min; and V_T = 2.12 ± 0.20 ml.

Calculations of TCE uptake and elimination were conducted according to equations presented in previous pharmacokinetic determinations of TCE inhalation in rats by this laboratory (Dallas et al., 1988). Since the V_E and the TCE exhaled breath concentration at each sampling point were measured, subtraction of the quantity of TCE exhaled from the animal from the amount inhaled yielded an approximation of the quantity of uptake of TCE for each sampling period (cumulative uptake). In the determination of the cumulative elimination of TCE during inhalation exposure, the contribution of inhaled compound in the dead space volume of the rat was subtracted from the concentration of chemical measured in the exhaled breath. The percent uptake of the total inhaled dose up to each successive time point during the inhalation exposure period was determined by dividing the cumulative uptake by the total inhaled dose for that time period.

A physiological pharmacokinetic model was used to describe the disposition of TCE in the rat (Fig. 1). It was assumed that a blood flow-limited model was adequate to characterize the tissue distribution

of TCE. Compartmental volumes and organ blood flows were obtained from Ramsey and Andersen (1984) and scaled to 340 g, the mean body weight of rats used in the present study. Partition coefficients for TCE and initial estimates of the Michelis-Menten parameters were taken from Andersen et al. (1987). The alveolar mass transfer coefficient was based on the alveolar permeability-area product for methylene chloride (Angelo and Pritchard, 1987). Differential mass balance equations, incorporating the parameters listed in Table 1, that described the transport of TCE in the rat were solved with the ACSL, Advanced Continuous Simulation Language, computer program (Mitchell and Gauthier, Concord, MA). The solution to the equations provided predicted TCE concentrations as a function of time.

Analysis of TCE in air and blood. The concentration of TCE in the inhaled and exhaled air during the inhalation exposures were measured with a Tracor MT560 gas chromatograph (Tracor Instruments, Austin, TX). Analyses for the 500 ppm inhalation exposures were conducted using a flame ionization detector (FID), while the analyses for the 50 ppm inhalation exposures were conducted using an electron capture detector (ECD). In either case, air samples were procured with a gas-tight, 1-ml syringe and injected directly onto an 8-ft X 1/8-in stainless-steel column packed with 0.1% AT 1000 on GraphPak. Standards were prepared in each of four 9-liter standard bottles which have Teflon® stoppers containing needles used for taking the air samples with the syringe. Operating temperatures were: 150°C, injection port; 200°C, FID detector; 350°C, ECD detector; 110°C, isothermal column operation. When using the ECD, gas flow rates were employed of 40 ml/min for nitrogen

(carrier gas), with an additional make-up gas flow rate to the detector of 30 ml/min.

TCE levels in the blood were measured by gas chromatographic headspace analysis. Blood samples were withdrawn from the arterial cannula via a stopcock by a 1-ml syringe. Depending on the anticipated blood concentration, between 25 and 200 µl of the blood was taken from the stopcock with an Eppendorf pipette and transferred to chilled headspace vials (Perkin-Elmer, Norwalk, CT). These vials were capped immediately with PTFE lined butyl rubber septa and washers and tightly crimped. Each sample vial was then placed into the HS-6 auto-sampler unit of a SIGMA 300 gas chromatograph (Perkin-Elmer, Norwalk, CT), where it was heated to a preset temperature by a high precision thermostat device. A precise volume of the vapor was then injected automatically into the column for analysis. The column used was an 8-ft X 1/8-in stainless-steel column packed with FFAP Chromasorb W-AW (80-100 mesh). Operating temperatures were: 250° C, injection port; 350° C, ECD detector; and column oven, 80° C. The carrier gas was 5% argon-methane, at a flow rate of 40 ml/min with a make-up gas flow rate to the detector of 20 ml/min.

RESULTS

While 50 and 500 ppm were the target TCE inhalation concentrations, the actual concentration inhaled by the animals was determined by measurements of air samples taken from the airway immediately adjacent to the breathing valve. Inhaled TCE concentrations for the six rats in

each group were 499.8 ± 12.7 ppm for the 500 ppm exposures and 50.7 ± 0.8 ppm for the 50 ppm exposures.

During and following TCE inhalation, concentrations of the parent compound were measured in the blood and exhaled breath of 50 ppm (Fig. 2) and 500 ppm (Fig. 3) exposed rats. Significant respiratory elimination of unchanged TCE was evident during the inhalation exposure period, with near steady-state TCE levels achieved in the exhaled breath within 20-30 min. TCE respiratory elimination was also proportional to the inhaled concentration, as indicated by the exhaled breath values during 30-120 min of the exposure period (near steady-state) of 34.6 ± 0.4 ppm and 340.8 ± 3.4 ppm (x ± SE) for the 50 and 500 ppm exposure groups, respectively. The concentration of TCE in the blood, however, progressively increased over the course of the 2-hr exposure in both exposure groups, with a higher rate of increase demonstrated in the 500 ppm-exposed rats relative to those that inhaled 50 ppm TCE. For example, blood levels after 120 min of exposure to 500 ppm TCE were 41% higher than after 30 min, while the 50 ppm group demonstrated a 22% increase over the same time period. Arterial TCE concentrations were not proportional to the inhalation concentration. After the initial rapid uptake phase over the first 30 minutes of exposure, blood levels for the 500 ppm-exposed rats were 25 to 30 times higher than TCE blood concentrations of rats that received 50 ppm exposures. Evaluation of the ratio of TCE concentration in the blood to TCE concentration in the exhaled breath over the duration of the inhalation exposure (Fig. 4) reveals a distinct difference between the two dose groups. This ratio increases only slightly over the 2 hr exposure for the 50 ppm group. For the rats inhaling 500 ppm TCE, this ratio increases rapidly from a

value near the initial point for the 50 ppm group to a level 3 times higher by the end of the exposure period.

Measurement of the cumulative uptake of TCE by the rats (Fig. 5) was made by accounting for the quantity of unchanged TCE that was exhaled during the inhalation exposure period. As a result of the 2-hr exposure to 500 ppm TCE the cumulative uptake was 24.3 ± 1.2 mg (x ± SE), or 73.3 mg/kg bw. The total cumulative uptake of TCE from the 2-hr exposure to 50 ppm was 2.96 ± 0.32 mg (x ± SE), or 8.4 mg/kg bw. While percent uptake of TCE during the first hour of inhalation exposure was initially higher for the 500 ppm group relative to rats inhaling 50 ppm TCE (Fig. 6), the difference was not statistically significant. Nearly complete uptake of the inhaled TCE in the first few minutes of exposure declined rapidly to a near steady-state equilibria during the second hour of the inhalation exposure period. Indeed, the uptake for both exposure groups were very similar during near-steady state, with values between approximately 69-72% in the second hour of exposure.

During the inhalation exposure, measurement of the cumulative elimination (Fig. 7) reflected both respiratory elimination of unchanged TCE from the systemic circulation and inhaled TCE that was expired from the physiologic dead space of the animal without participating in pulmonary absorption. By the end of the 2-hr exposure to 50 and 500 ppm TCE, 1.33 ± 0.22 and 11.59 ± 2.11 mg (x ± SE), respectively, of unchanged TCE were eliminated in the exhaled breath of the exposed rats. Continued elimination of TCE following the termination of exposure resulted solely from the exhalation of unmetabolized TCE from the lung following transfer across the alveolocapillary membrane from the systemic circulation. For example, after the first hour immediately

following termination of the exposure, 0.17 and 3.4 mg of unchanged TCE was eliminated from the systemic circulation of the rats that received 50 and 500 ppm TCE exposures, respectively. Therefore, while the cumulative respiratory elimination of TCE during exposure (including both unabsorbed and absorbed-and-eliminated TCE) was approximately proportional to the inhalation concentration, post-exposure cumulative elimination of unchanged TCE solely from the systemic circulation was not.

Figures 2 and 3 show the observed and model predicted TCE blood an exhaled breath concentrations for the 50 and 500 ppm exposures, respectively. In general, the predictions are in agreement with the actual data, with one model overpredicting blood concentrations during the 50 ppm exposure and the exhaled breath concentration during the 500 ppm exposure. These overpredictions are on the order of 0.1 µg/ml. From 180 min, all predicted values are in excellent agreement with the observed TCE concentrations.

DISCUSSION

Direct measurements of the pharmacokinetics of a chemical provides unique and important information that facilitates species to species extrapolations. For volatile organic solvents, such measurements should include monitoring of the levels of the compound in the exhaled breath during inhalation exposure, as such information is essential to defining both the absorbed dose during inhalation exposures as well as the

relative respiratory elimination of the compound. Simultaneous direct determinations of the parent compound in the blood during solvent inhalation provides a quantitatve indicator of the systemic uptake, disposition and metabolism of the parent compound. When such comparable pharmacokinetic data is available in both humans and test animal species, it provides a more definitive basis for validating the interspecies extrapolations of toxicity data. Such correlative knowledge of the uptake, disposition, and elimination of environmental contaminants in interspecies comparisons has become crucial in the formulation of accurate health risk assessments. Ironically, while some direct pharmacokinetic determinations of inhaled TCE are available in humans, there has previously been a lack of direct measurements of TCE in the exhaled breath and blood of laboratory animals during inhalation exposures to TCE.

Utilizing the direct measurements of TCE in the inhaled and exhaled breath of rats in the current investigation, the total cumulative uptake of TCE during a 2 hr inhalation exposure was determined (i.e. 73.3 mg/kg for the 500 ppm dose group). In a previous pharmacokinetic study of TCE inhalation in rats involving 600 ppm exposures to ^{14}C-TCE for 6 hr, a total body burden of 141 mg/kg was calculated from determinations of the recovery of radioactivity (Stott et al., 1982). When the cumulative uptake value for the 500 ppm exposure group is adjusted for variations in rat weight, exposure level, and length of inhalation, a very similar value of 143 mg/kg is obtained for rats in the present study. In humans, an uptake of inhaled TCE of 6.6 mg/kg has been reported after 4 hr of exposure to 70 ppm (Monster et al., 1979). When adjusted for minor differences in exposure level and duration, this is approximately

one-fourth of the cumulative uptake seen in the 50 ppm exposures in rats in the current study. Further pharmacokinetic differences between rats and man are also apparent in the pattern of the absorbed dose of inhaled TCE relative to the inhaled concentration. In studies of TCE inhalation in humans, the dose absorbed was proportional to the level of TCE inhaled (Muller et al., 1974; Monster et al., 1976, 1979; Fernandez et al., 1977). These results have been interpreted in health risk assessments of TCE that there is not sufficient evidence to indicate saturation of TCE metabolism occurs in man (EPA, 1985). In previous studies of inhaled TCE in rats, however, a 60-fold increase in inhaled concentration was accompanied by an increase of only 30 times in total body uptake of ^{14}C-TCE (Stott et al., 1982). In the present study of TCE inahaltion in rats, an 8.7-fold difference in the cumulative uptake was seen between the 50 and 500 ppm dose groups. Additionally, TCE blood levels during the second hour of exposure (after the rapid uptake phase) for the 500 ppm group were approximately 25 times that of the rats that received 50 ppm exposure. From indirect measurements involving the disappearance of TCE from closed jars containing rats inhaling the solvent, Filser and Bolt (1979) have calculated that the point of saturation for the metabolism of inhaled TCE in Wistar rats is 65 ppm (350 mg/m^3). It is apparent from the direct measurements during TCE inhalation in the present study that TCE metabolism is saturable in the Sprague-Dawley rat in a dose-dependent manner between 50 and 500 ppm TCE inhalation.

While respiratory elimination and blood concentration data during TCE inhalation has been lacking in laboratory animals, there have been several studies involving these determinations post-exposure in humans.

The exhaled breath of exposed workers has been monitored for expired TCE following inhalation exposure as a non-invasive method for indicating the magnitude of prior exposure to the solvent (Stewart et al., 1970, 1974). Measurements of TCE in both the blood and exhaled breath of workers following TCE inhalation have been made in studies of the effect of workload (Monster et al., 1976) and repeated exposure to TCE (Monster et al., 1979) on subsequent pharmacokinetics of the inhaled solvent. Accounting for differences in exposure concentration, the post-exposure exhaled breath levels of TCE from these studies in humans were similar in magnitude to the values of TCE eliminated in the exhaled breath of rats following inhalation exposure in the current investigation. For instance, Stewart et al. (1974) found human exhaled TCE levels of 0.70 and 0.28 ppm at 30 and 120 min, respectively, after termination of 20 ppm TCE inhalation for 3 hrs. Scaling-down the 50 ppm data in rats in the present study at these time points would yield 0.92 and 0.28 ppm, respectively.

A similar adjusted comparison of TCE blood level data at the termination of exposure reveals a similarity in magnitude of the blood concentrations in the 50 ppm TCE-dosed rats and those in humans after 4 hr inhalation to 70 ppm (Monster et al., 1979), though such comparisons are inexact due to the different lengths of exposure. However, such interspecies similarities in blood levels are probably only relevant involving comparisons at doses below that at which metabolic saturation is occurring in rats. The ratio of arterial TCE level to inspired air concentration for a 30 min exposure to 100 and 200 ppm TCE in humans has been reported to be 215% (Astrand, 1975), which is comparable to the 185% value seen in the current study for 30 min of exposure to 50 ppm in

rats. However, the value in rats for 500 ppm exposure is 458%, an indicator that metabolic saturation has occurred within the first 30 min of exposure to the higher dose. From a review of the various studies of the metabolism of inhaled TCE in man, it has been concluded that there was not sufficient evidence to indicate that saturation of TCE metabolism occurs in man (EPA, 1985). However, there were apparent dose-dependent differences in TCE metabolism reflected by relatively increased urinary excretion of the trichloroacetic acid metabolite in humans exposed to greater than 50 ppm TCE inhalation (Ikeda et al., 1972). Other studies examining the dose-dependence of urinary metabolite excretion relative to inhaled TCE concentration (Nomiyama and Nomiyama, 1977; Ikeda, 1977) seem to indicate that metabolism of inhaled TCE in unlimited up to highest dose employed (315 ppm for 3 hr, or an inhaled dose of 25 mg TCE/kg bw). If saturation of TCE metabolism does not occur in man, it is reasonable to assume that interspecies comparisons of TCE blood levels between rats and man at higher exposure levels (i.e. 500 ppm) will result in considerably higher blood levels in rats relative to man.

Another advantage of direct measurements of test compounds in pharmacokinetic studies is their utility in the development of physiologically-based pharmacokinetic (PBPK) models. The present model incorporated the dynamics between the venous, alveolary and arterial compartments that has been used for a methylene chloride PBPK model (Angelo and Pritchard, 1984, 1987). The representation is appealing in that venous and arterial blood pools are distinct, and a physiologically realistic membrane transport term (h) controls chemical uptake and elimination at the alveolar-lung interface. The blood flow-limited

tissue compartments and the Michelis-Menten liver elimination are similar to other models on metabolized volatile organic compounds (Andersen et al., 1987). The experimentally measured model parameters, V_a and the inhaled gas concentration, were the only values that were altered for the predictions obtained at the 50 and 500 ppm exposures. Tissue TCE concentration would be of great use in potential revisions of the model and in validating it.

REFERENCES

ANDERSEN, M. E., GARGAS, M. L., CLEWELL, H. J., AND SEVERYN, K. M. (1987). Quantitative evaluation of the metabolic interactions between trichloroethylene and 1,1-dichloroethylene in vivo using gas uptake methods. Toxicol. Appl. Pharmacol. 89, 149-157.

ANDERSEN, M. E., GARGAS, M. L., JONES, R. A., AND JENKINS, L. J. (1980). Determination of the kinetic constants for metabolism of inhaled toxicants in vivo using gas uptake measurements. Toxicol. Appl. Pharmacol. 54, 100-116.

ANGELO, M. J. AND PRITCHARD, A. B. (1987). Route-to-route extrapolation of dichloromethane exposure using a physiological pharmacokinetic model. In Pharmacokinetics in Risk Assessment, Drinking Water and Health, Vol. 8, National Academy Press, Washington, DC.

ANGELO, M. J. AND PRITCHARD, A. B. (1984). Simulations of methylene chloride pharmacokinetics using a physiologically based model. Reg. Toxicol. Pharmacol. 4, 329-339.

ASTRAND, I. (1975). Uptake of solvents in the blood and tissues of man. A review. Scand. J. Work Environ. Health. 1, 199-218.

ASTRAND, I., KILBOM, A., WAHLBERG, I., AND OVRUM, P. (1973). Methyl-chloroform exposure. I. Concentration in alveolar air and blood at rest and during exercise. Work Environ. Health 9, 69-81.

DALLAS, C. E., WEIR, F. W., FELDMAN, S., PUTCHA, L., AND BRUCKNER, J. V. (1983). "The uptake and disposition of 1,1-dichloroethylene in rats during inhalation exposure". Toxicol. Appl. Pharmacol. 68, 140-151.

DALLAS, C. E., BRUCKNER, J. V., MAEDGEN, J. L., AND WEIR, F. W. (1986). "A method for direct measurement of systemic uptake and elimination of volatile organics in small animals." J. Pharmacol. Methods 16, 239-250.

DEFALQUE, R. J. (1965). Pharmacology and Toxicology of Trichloroethylene. Clin. Pharmacol. Ther. 2, 655-688.

D'SOUZA, R. W., BRUCKNER, J. V. AND FELDMAN, S. (1985). Oral and intravenous pharmacokinetics of trichloroethylene in rats. J. Toxicol. Environ. Health 15, 587-601.

Environmental Protection Agency (1985). Health assessment document for trichloroethylene. EPA/600/8-82-006. U.S. Environmental Protection Agency, Washington, DC.

FERNANDEZ, J. G., DROZ, P. O., HUMBERT, B. E. AND CAPEROS, J. R. (1977). Trichloroethylene exposure. Simulation of uptake, excretion, and metabolism using a mathematical model. Brit. J. Ind. Med. 34, 43-55.

FILSER, J. G. AND BOLT, H. M. (1979). Pharmacokinetics of halogenated ethylenes in the rat. Arch. Toxicol. 42, 123-136.

GANONG, W. F. (1979). Review of Medical Physiology, p. 500. Lange Medical Publications, Los Altos, California.

GARGAS, M. L., ANDERSEN, M. E., AND CLEWELL, H. J., III (1986). A physiologically-based simulation approach for determining metabolic constants from gas uptake data. Toxicol. Appl. Pharmacol. 86, 341-352.

IKEDA, M. (1977). Metabolism of trichloroethylene and tetrachloroethylene in human subjects. Environ. Health Persp. 21, 239-245.

IKEDA, M., OHTSUJI, H., IMAMURA, T. AND KOMOIKE, Y. (1972). Urinary excretion of total trichloro-compounds, trichloroethanol, and trichloroacetic acid as a measure of exposure to trichloroethylene and tetrachloroethylene. Brit. J. Ind. Med. 29, 328-333.

LONGLEY, E. D. AND JONES, R. (1963). Acute Trichloroethylene Narcosis. Arch. Environ. Health. 7, 249-253.

MONSTER, A. C., BOERSMA, G., DUBA, W. C. (1976). Pharmacokinetics of trichloroethylene in volunteers; influence of work load and exposure concentration. Int. Arch. Occup. Environ. Health. 38, 87-102.

MONSTER, A. C., BOERSMA, G., AND DUBA, W. C. (1979). Kinetics of trichloroethylene in repeated exposure of volunteers. Int. Arch. Occup. Environ. Health 42, 283-292.

MULLER, G., SPASSOVSKI, M. AND HENSCHLER, D. (1974). Metabolism of trichloroethylene in man. II. Pharmacokinetics of metabolites. Arch. Toxicol. 32, 283-295.

NAKAJIMA, T., OKINO, T., OKUYAMA, S. KANEKO, T., YONEKURA, I. AND SATO, A.(1988). Ethanol-induced enhancement of trichloroethylene metabolism and hepatotoxicity: difference from the effect of phenobarbital.
Toxicol. Appl. Pharmacol. 94, 227-237.

National Cancer Institute (1976). Carcinogenesis bioassay of trichloroethylene. C.A.S. No. 79-01-6. D.H.E.W. Publ. No. (NIH) 76-802.

National Institute for Occupational Safety and Health (1973). Criteria for a recommended standard-Occupational exposure to trichloroethylene. DHEW publ. No. HSM-73-11025, National Institute for Occupational Safety and Health, Washington, DC.

National Institute for Occupational Safety and Health (1978). Special occupational hazard review of trichloroethylene. DHEW publ. No. 78-130, National Institute for Occupational Safety and Health, Washington, DC.

National Toxicology Program (1983). NTP technical report on the carcinogenesis bioassay of trichloroethylene in F344/N rats and B6CF1/N mice. NIH publ. No. 83-1799, National Toxicology Program, Research Triangle Park, NC and Bethesda, MD.

NOMIYAMA, K. AND NOMIYAMA, H (1971). Metabolism of trichloroethylene in humans. Sex difference in urinary excretion of trichloroacetic acid and trichloroethanol. Intl. Arch. Arbeitsmed. 28, 37-48.

RAMSEY, J. C. AND ANDERSEN, M. E. (1984). A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. Toxicol. Appl. Pharmacol. 73, 159-175.

STEWART, R. D., HAKE, C. L. AND PETERSON, J. E. (1974). Use of breath analysis to monitor trichloroethylene exposures. Arch. Environ. Health 29, 6-13.

STEWART, R. D., DODD, H. C.,GAY, H. H. AND ERLEY, D. S. (1970). Experimental human exposure to trichloroethylene. Arch. Environ. Health 20, 64-71.

STOTT, W. T., QUAST, J. F. AND WATANABE, P. G. (1982). The pharmacokinetics and macromolecular interactions of trichloroethylene in mice and rats. Toxicol. Appl. Pharmacol. 62, 137-151.

VENG-PEDERSEN, P. (1984). Pulmonary absorption and excretion of compounds in the gas phase: Theoretical pharmacokinetic and toxicokinetic analysis. J. Pharm. Sci. 73, 1136-1141.

WILLIAMS, J. W. (1959). The toxicity of trichloroethylene. J. Occup. Med. 1, 549-554.

WITHEY, J. R. AND COLLINS, B. T. (1980). Chlorinated aliphatic hydrocarbons used in the foods industry: the comparative pharmacokinetics of methylene chloride, 1,2-dichloroethane, chloroform, and trichloroethylene after i.v. administration in the rat. J. Environ. Pathol. Toxicol. 3, 313-332.

Figure Legends

1. Diagram of the physiological pharmacokinetic model used to simulate the uptake and elimination of inhaled TCE. The symbols and parameters used to describe the model are in Table I.
2. Observed (•) and model-predicted (-) TCE concentrations in the blood (top graph) and exhaled breath (bottom graph) of rats during and following a 2-hr 50 ppm inhalation exposure. Each observed value represents the mean for 6 rats.
3. Observed (•) and model-predicted (-) TCE concentrations in the blood (top graph) and exhaled breath (bottom graph) of rats during and following a 2 hr 500 ppm inhalation exposure. Each observed value represents the mean for 6 rats.
4. Ratio of the TCE arterial blood concentration to the TCE exhaled breath concentration at each sampling time point during inhalation exposure to TCE. Rats inhaled 50 or 500 ppm TCE for 2 hr. Each line reflects the best-fit regression equation for each dose group. Each observed value represents the mean ratio for 6 rats.
5. Cumulative uptake of TCE during inhalation exposures. Rats inhaled 50 or 500 ppm TCE for 2 hr. The quantity of inhaled TCE retained during successive 10-min intervals was calculated on the basis of the measured minute volume and difference between inhaled and exhaled TCE concentrations. Each point represents the mean ± SE for 6 rats.
6. Percent uptake of TCE during inhalation exposures. Rats inhaled 50 or 500 ppm TCE for 2 hr. Each point represents the mean ± SE for 6 rats. The percent uptake of the inhaled dose over time was

determined after 1, 3, 5, 10, 15, and 20 min and at 10-min intervals thereafter.

7. Cumulative elimination of TCE during and following inhalation exposures. Rats inhaled 50 or 500 ppm TCE for 2 hr. The quantity of inhaled TCE eliminated in the breath over time was calculated using direct measurements of the minute volume and TCE concentrations in the inhaled and exhaled breath. The contribution of inhaled TCE from instrumental and anatomic dead space to the quantity exhaled was deleted. Each point is the mean ± SE for 6 rats. Cumulative elimination was determined for successive 10-min intervals during the 2-hr exposure, and for successive 15-min intervals post-exposure.

FOOTNOTES

1 Research sponsored by U.S. EPA Cooperative Agreement CR 812267 and the Air Force Office of Scientific Research, Air Force Systems Command, USAF, under grant number AFOSR 87-0248. The US Government is authorized to reproduce and distribute reprints for Governmental purposes notwithstanding any copyright notation thereon. This manuscript is submitted for publication with the understanding that the US Government is authorized to reproduce and distribute reprints for Governmental purposes.

2 Presented at the 26th Annual Meeting of the Society of Toxicology, Washington, DC, February, 1987.

3 To whom correspondence should be addressed.

ACKNOWLEDGEMENT

The authors are grateful to Ms. Linda Quigley, Miss Elizabeth Lehman and Mrs. Judy Bates for their expertise in preparation of this manuscript.

INDEX TERMS

1,1,1-Trichloroethylene

Saturable metabolism

Physiologically-based Pharmacokinetic Model

Respiratory Elimination

Pharmacokinetics

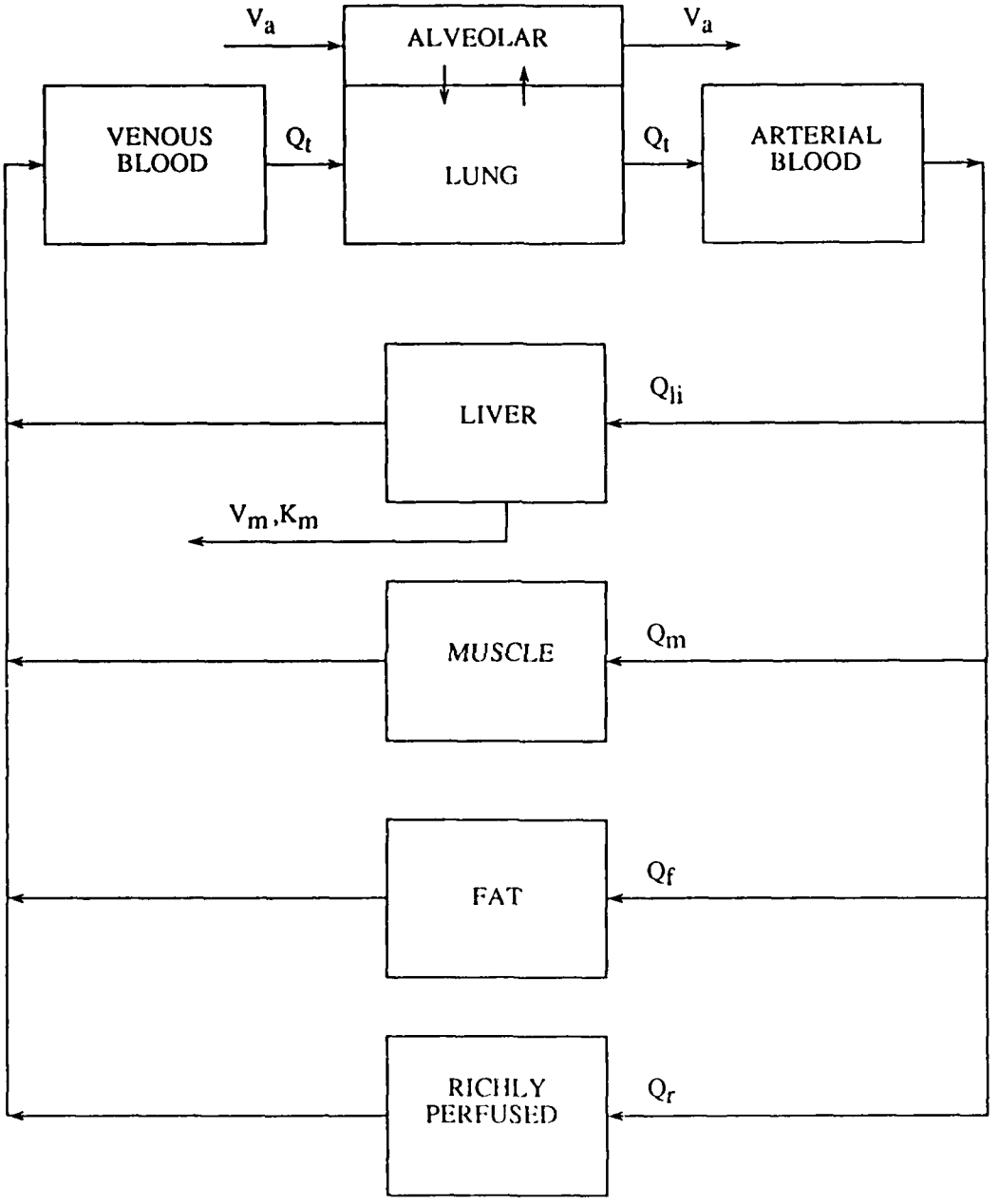
Inhalation Exposure

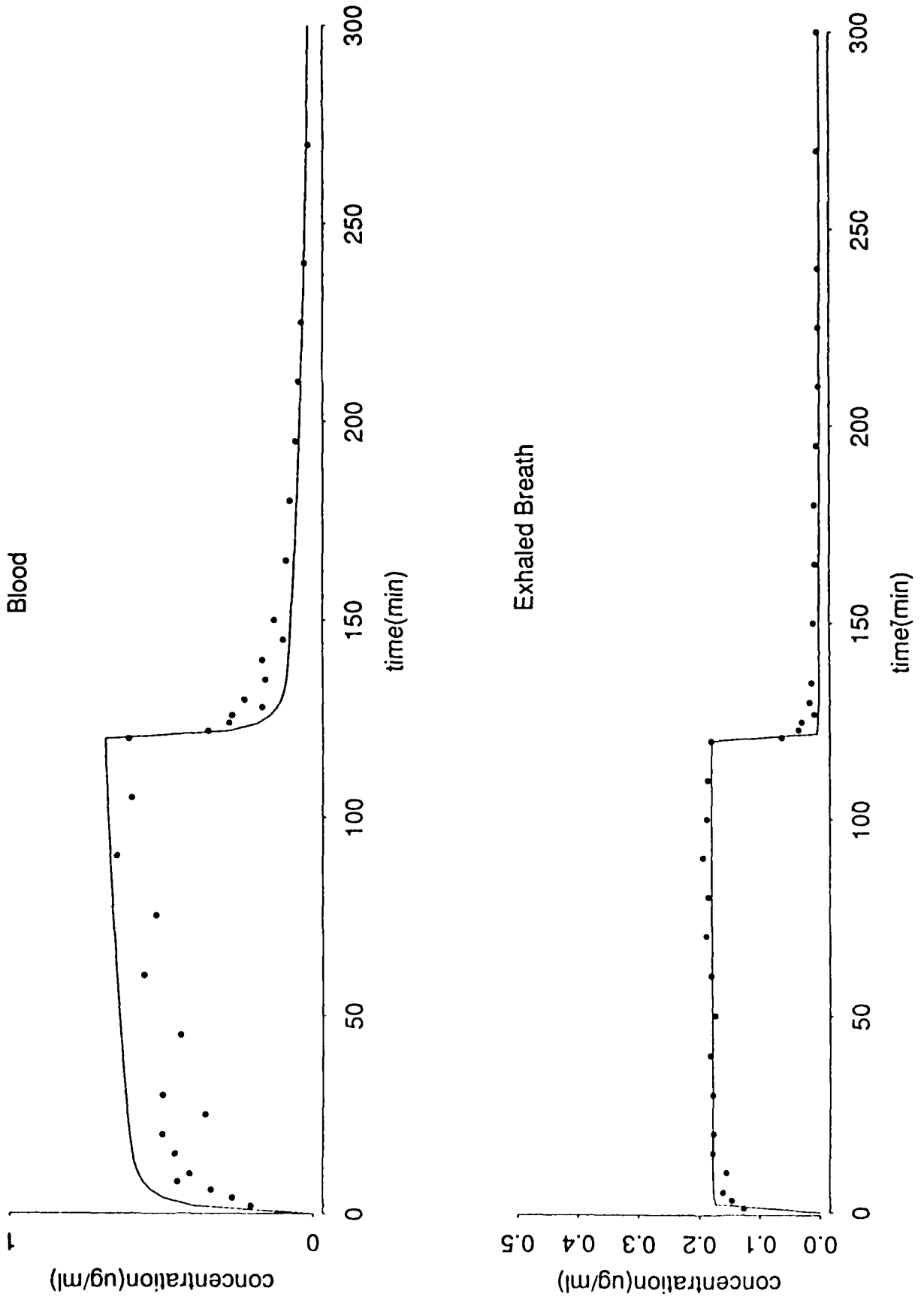
Interspecies Extrapolations

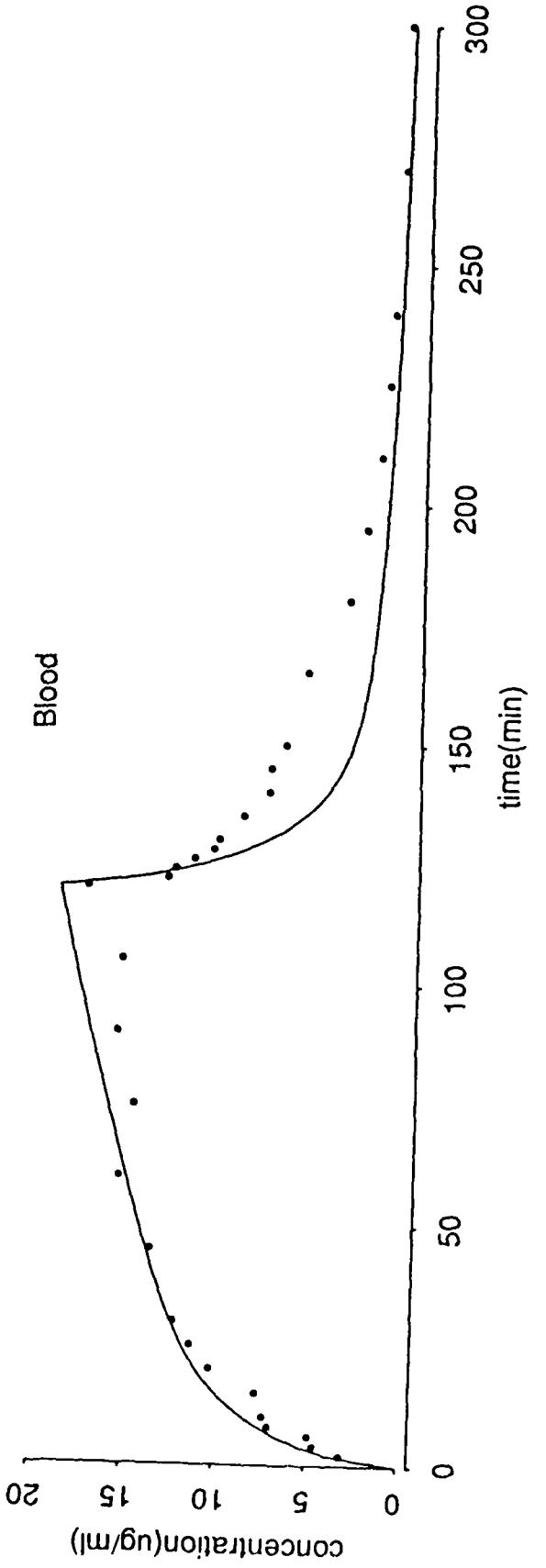
Table 1. Parameters for the Physiological Pharmacokinetic Model of TCE in the Rat (340 g)

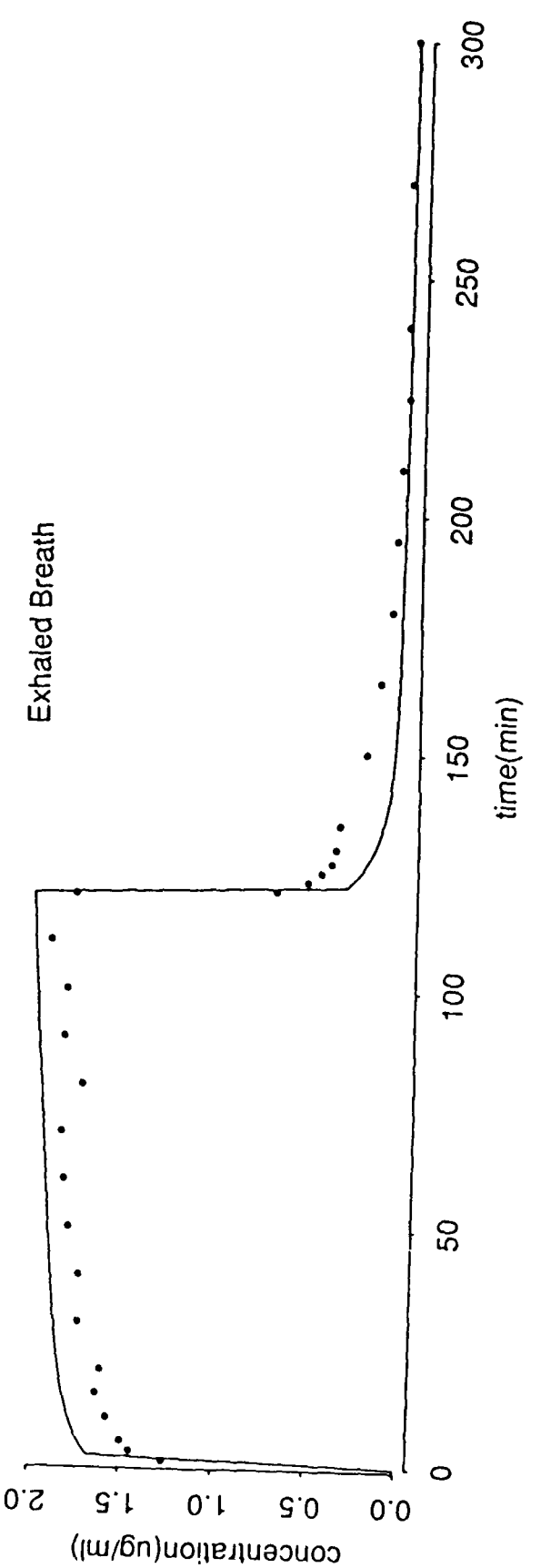
Parameter	Value
Alveolar Ventilation Rate (ml/min), V_a	109 (50 ppm exposure)
	134.5 (500 ppm exposure)
Inhaled Gas Concentration(µg/ml)	0.272 (50 ppm exposure)
	2.69 (500 ppm exposure)
Alveolar Mass Transfer Coefficient	500 ml/min
Blood Flows (ml/min)	
Cardiac output, Q_b	106.4
Fat, Q_f	9.4
Liver, Q_{li}	39.8
Muscle, Q_m	12.8
Richly Perfused, Q_r	44.4
Tissue Volumes (ml)	
Alveolar	2.0
Blood	25.4
Fat	30.5
Liver	13.6
Lung	3.97
Muscle	248.0
Richly Perfused	15.2
Partition Coefficients	
Blood:Air	21.9
Fat:Blood	25.3
Liver:Blood	1.24
Muscle:Blood	0.46
Richly Perfused: Blood	1.24
Metabolism Constants	
Vmax (ug/min)	75.0
Km (ug/ml)	0.25

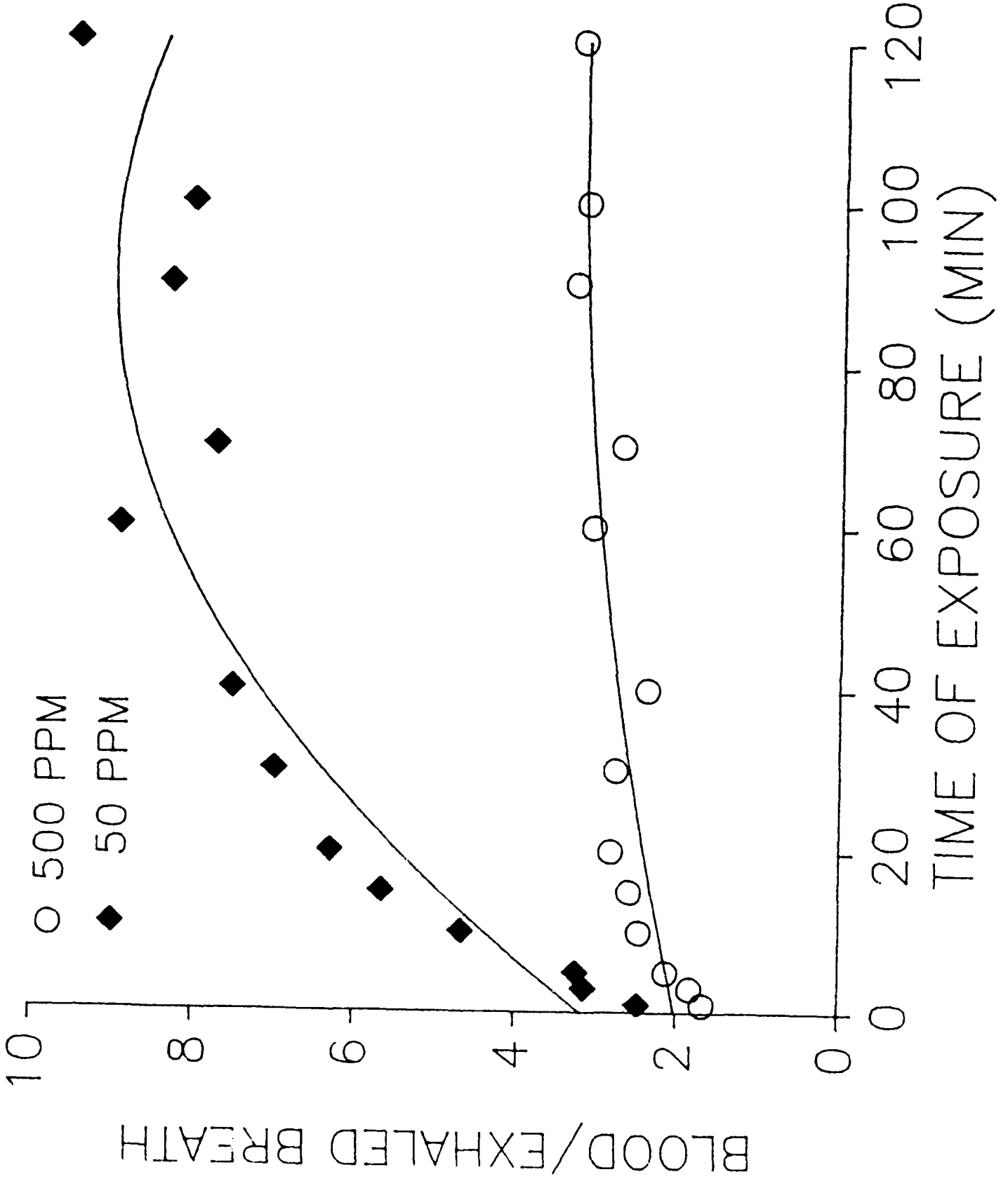
PPKM TCE

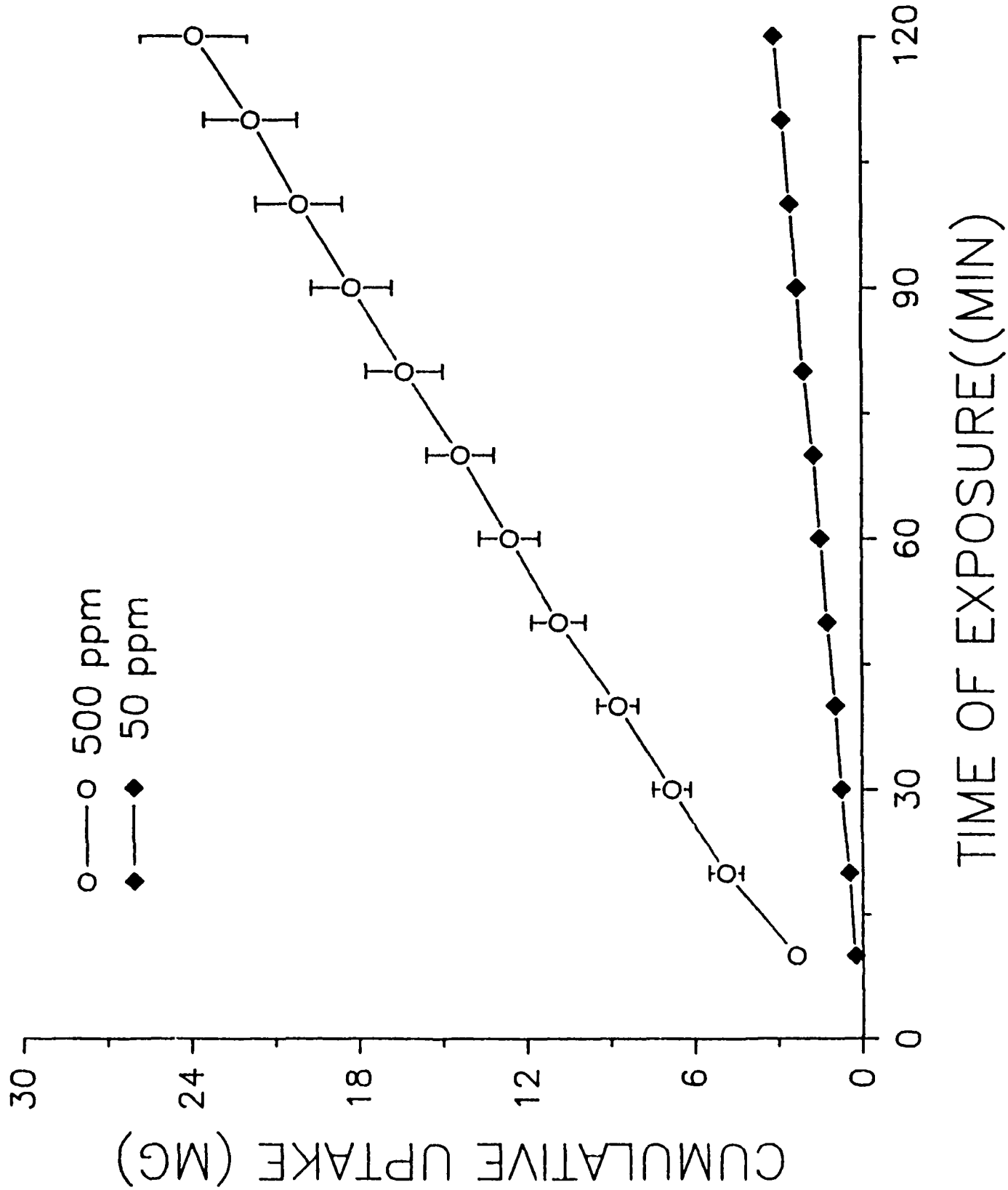

V_a
ALVEOLAR
V_a
VENOUS BLOOD
Q_t
LUNG
Q_t
ARTERIAL BLOOD
LIVER
Q_{li}
V_m, K_m
MUSCLE
Q_m
FAT
Q_f
RICHLY PERFUSED
Q_r

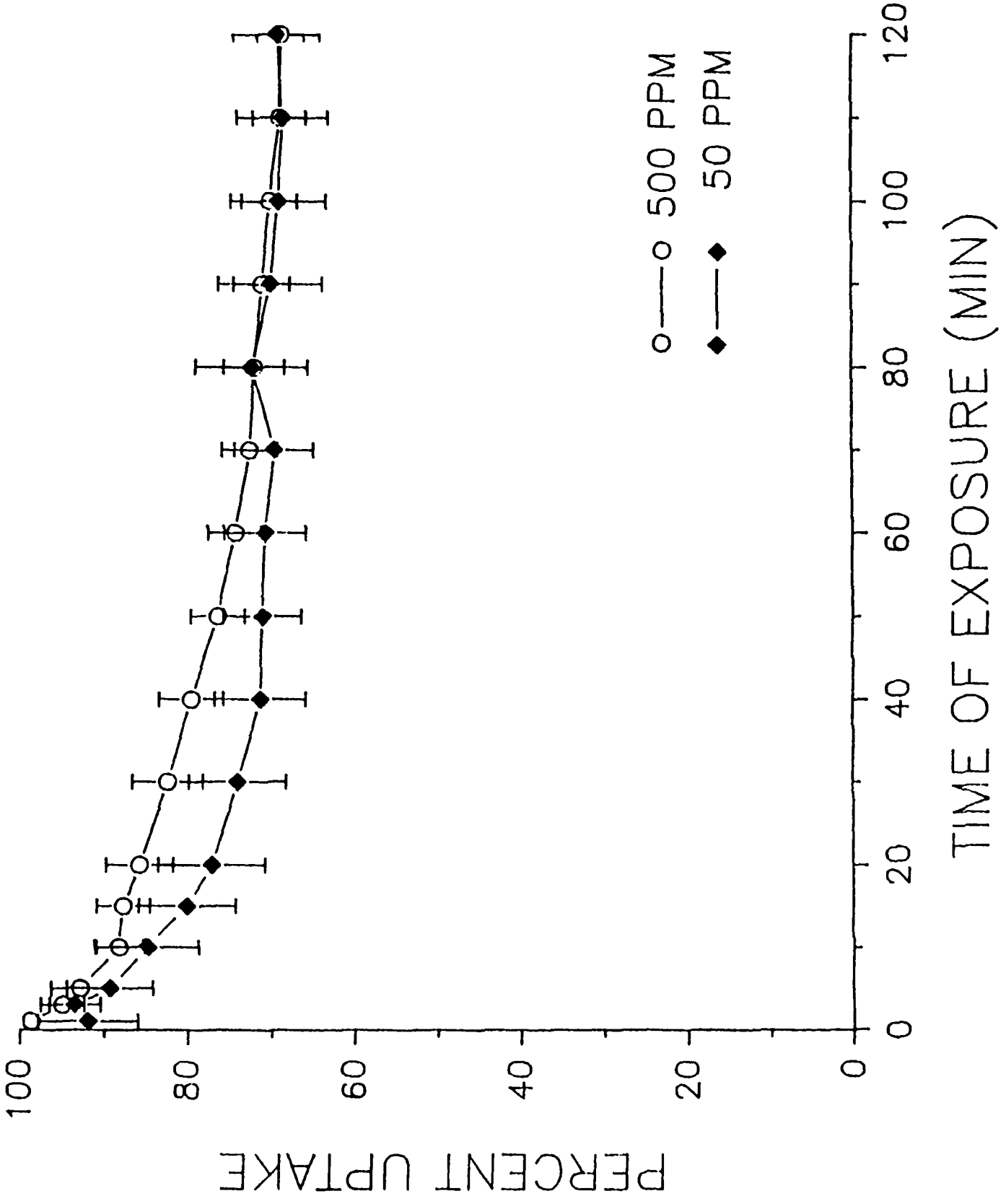

Blood
concentration(ug/ml)
1
0
0
50
100
150
200
250
300
time(min)
Exhaled Breath
concentration(ug/ml)
0.0
0.1
0.2
0.3
0.4
0.5
0
50
100
150
200
250
300
time(min)

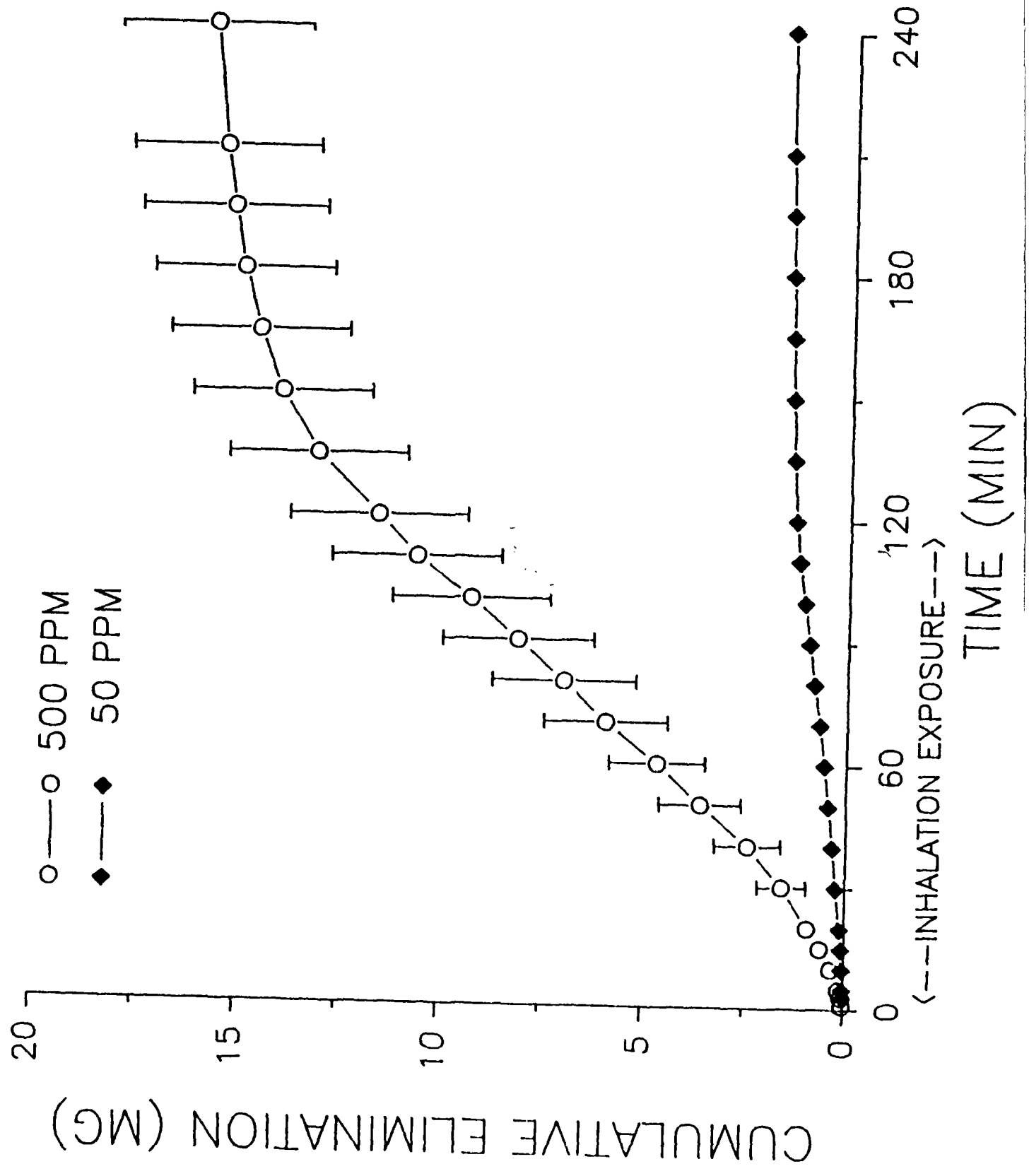

Blood
concentration(ug/ml)
0
5
10
15
20
0
50
100
150
200
250
300
time(min)


Exhaled Breath
concentration(ug/ml)
0.0
0.5
1.0
1.5
2.0
0
50
100
150
200
250
300
time(min)


BLOOD/EXHALED BREATH
TIME OF EXPOSURE (MIN)
0
20
40
60
80
100
120
2
4
6
8
10
500 PPM
50 PPM


CUMULATIVE UPTAKE (MG)
30
24
18
12
6
0
0
30
60
90
120
TIME OF EXPOSURE((MIN)
500 ppm
50 ppm


PERCENT UPTAKE
100
80
60
40
20
0
0
20
40
60
80
100
120
TIME OF EXPOSURE (MIN)
500 PPM
50 PPM


CUMULATIVE ELIMINATION (MG)
20
15
10
5
0
0
60
120
180
240
TIME (MIN)
<--INHALATION EXPOSURE-->
o—o 500 PPM
◆—◆ 50 PPM

APPENDIX C

ADDITIONAL DATA INCLUDED IN FIRST ANNUAL REPORT

(now being prepared for manuscript submission)

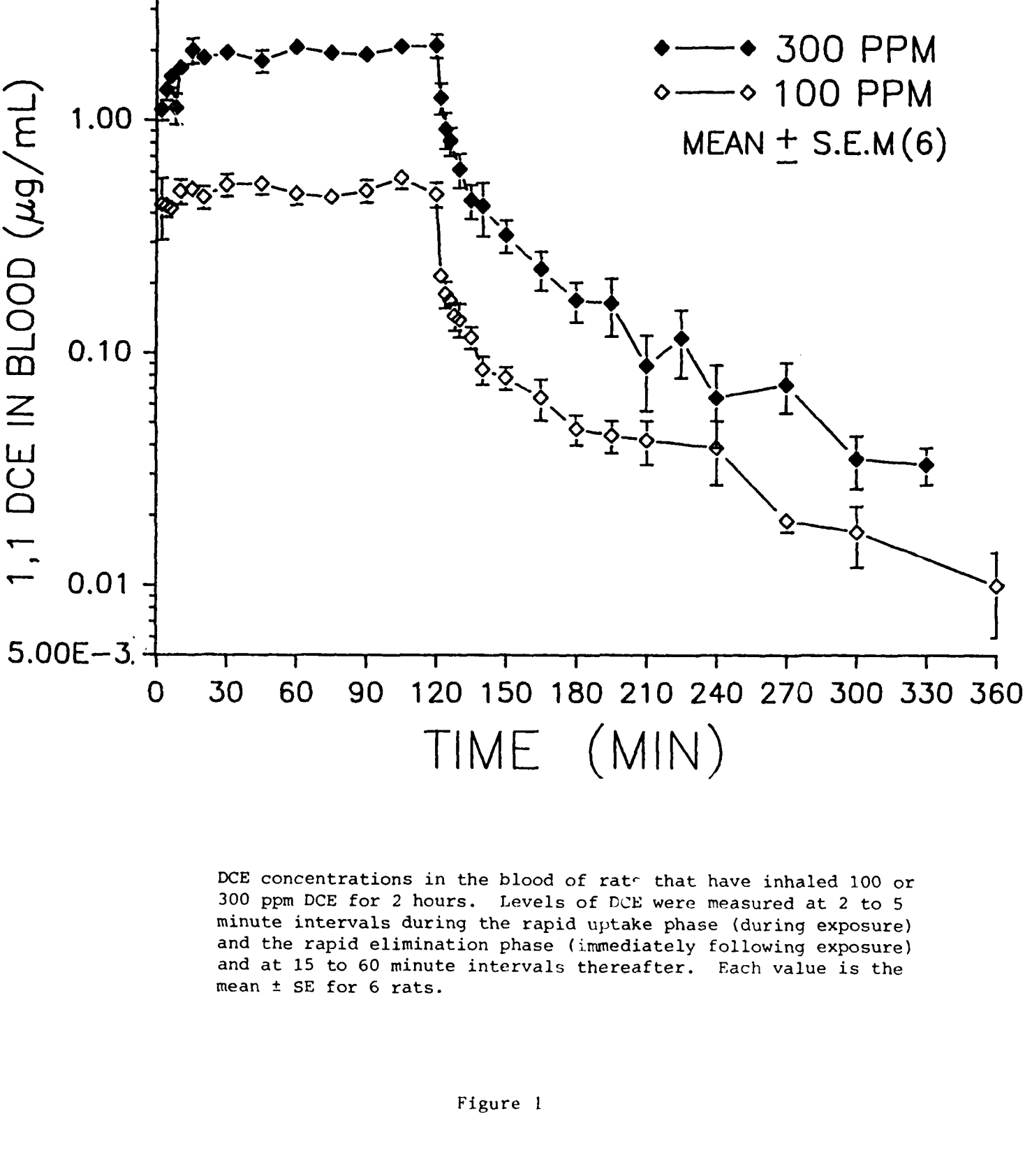
1.) DCE Inhalation Pharmacokinetics in Rats

2.) DCE Oral Pharmacokinetics in Rats

3.) TRI Oral Pharmacokinetics in Rats

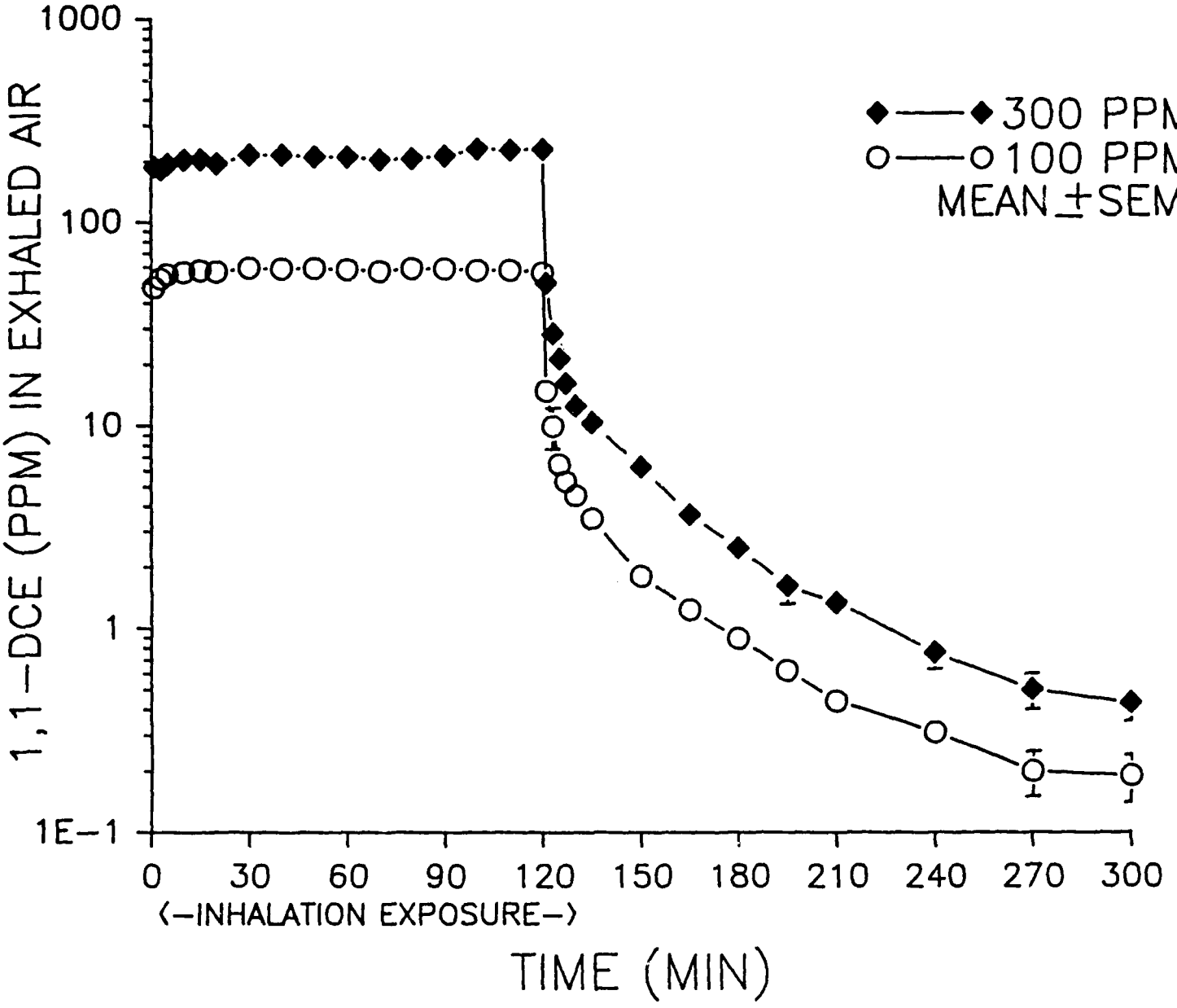
4.) TCE Oral Pharmacokinetics in Rats

5.) Physiologically-based Pharmacokinetic Model Simulations of Ingested TRI in Rats



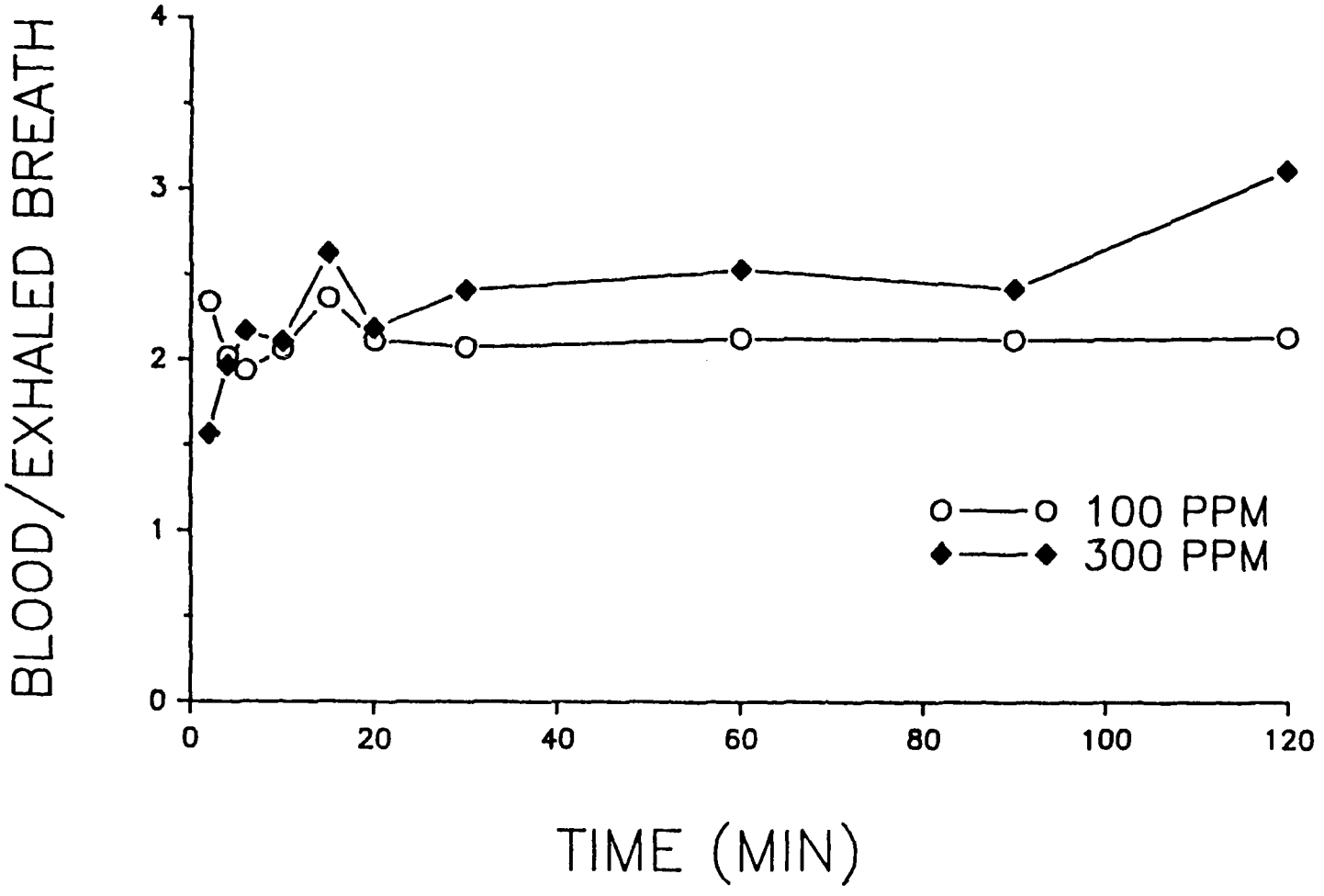
DCE concentrations in the blood of rats that have inhaled 100 or 300 ppm DCE for 2 hours. Levels of DCE were measured at 2 to 5 minute intervals during the rapid uptake phase (during exposure) and the rapid elimination phase (immediately following exposure) and at 15 to 60 minute intervals thereafter. Each value is the mean ± SE for 6 rats.

Figure 1



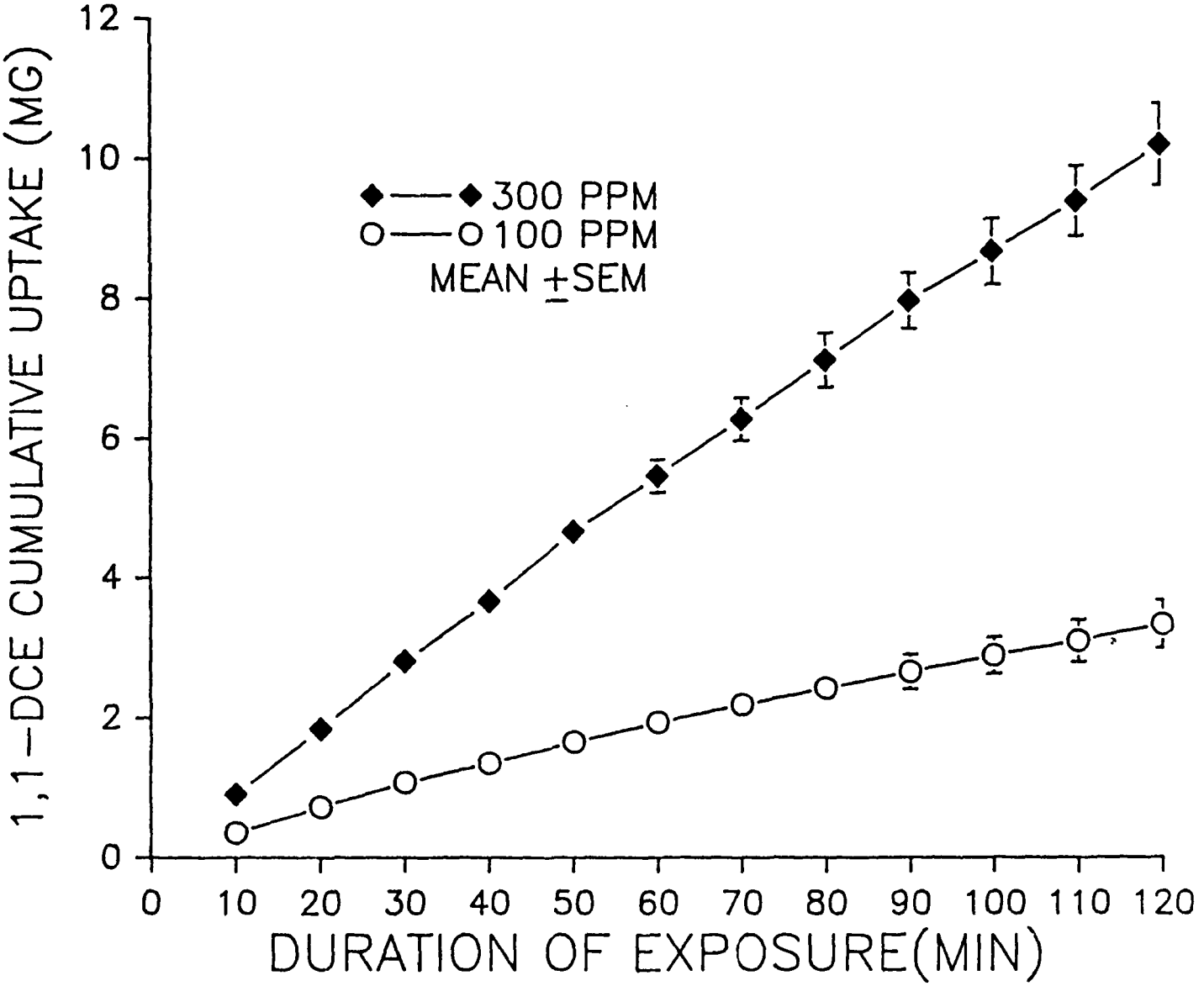
DCE concentrations in the exhaled breath of rats that have inhaled 100 or 300 ppm DCE for 2 hours. Levels of DCE were measured at 2 to 5 minute intervals during the rapid uptake phase (during exposure) and the rapid elimination phase (immediately following exposure) and at 15 to 60 minute intervals thereafter. Each value is the mean ± SE for 6 rats.

Figure 2



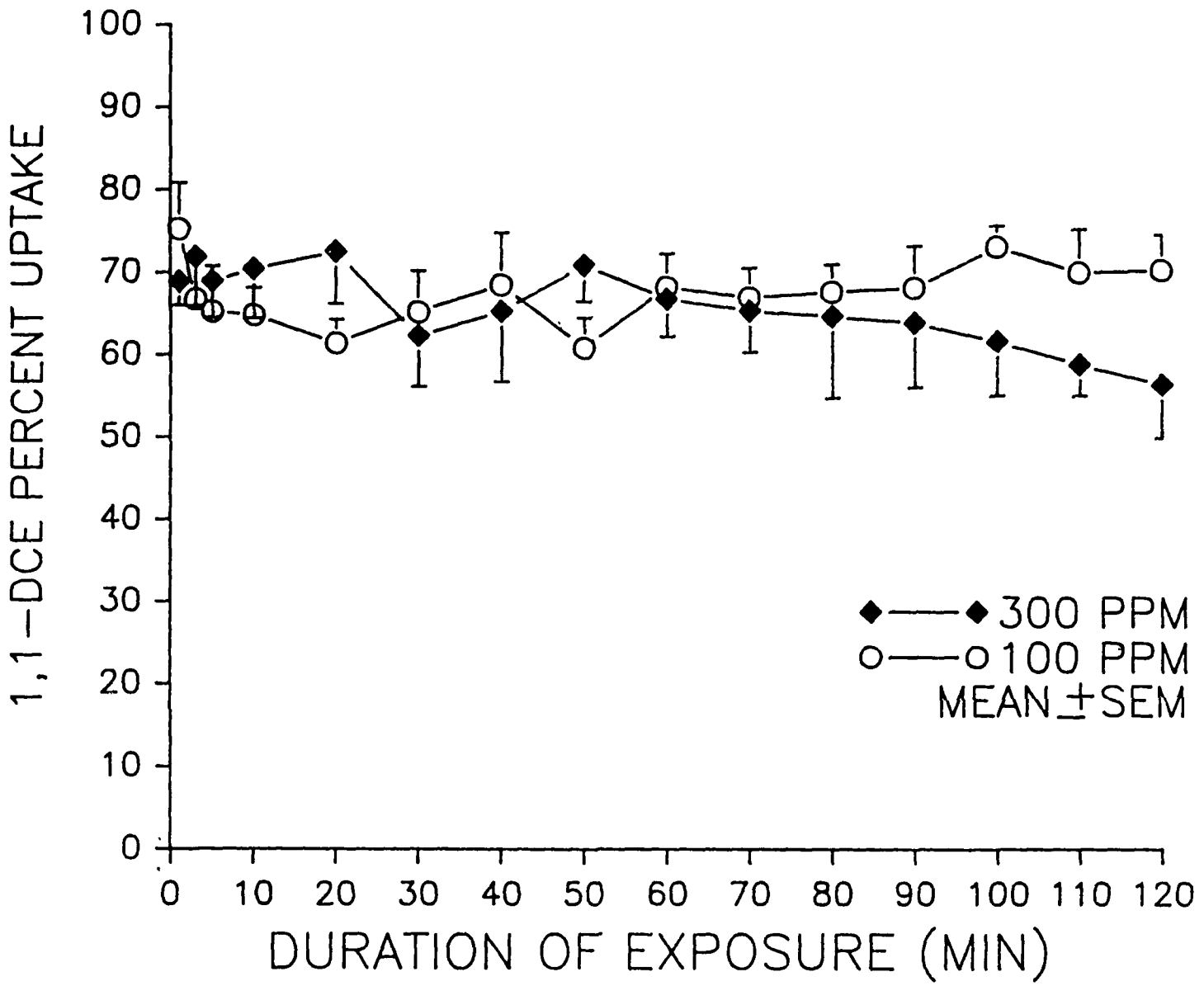
Ratio of the DCE arterial blood concentration to the DCE exhaled breath concentration at each sampling time point during inhalation exposure to DCE. Rats inhaled 100 or 300 ppm DCE for 2 hr. Each observed value represents the mean ratio for 6 rats.

Figure 3



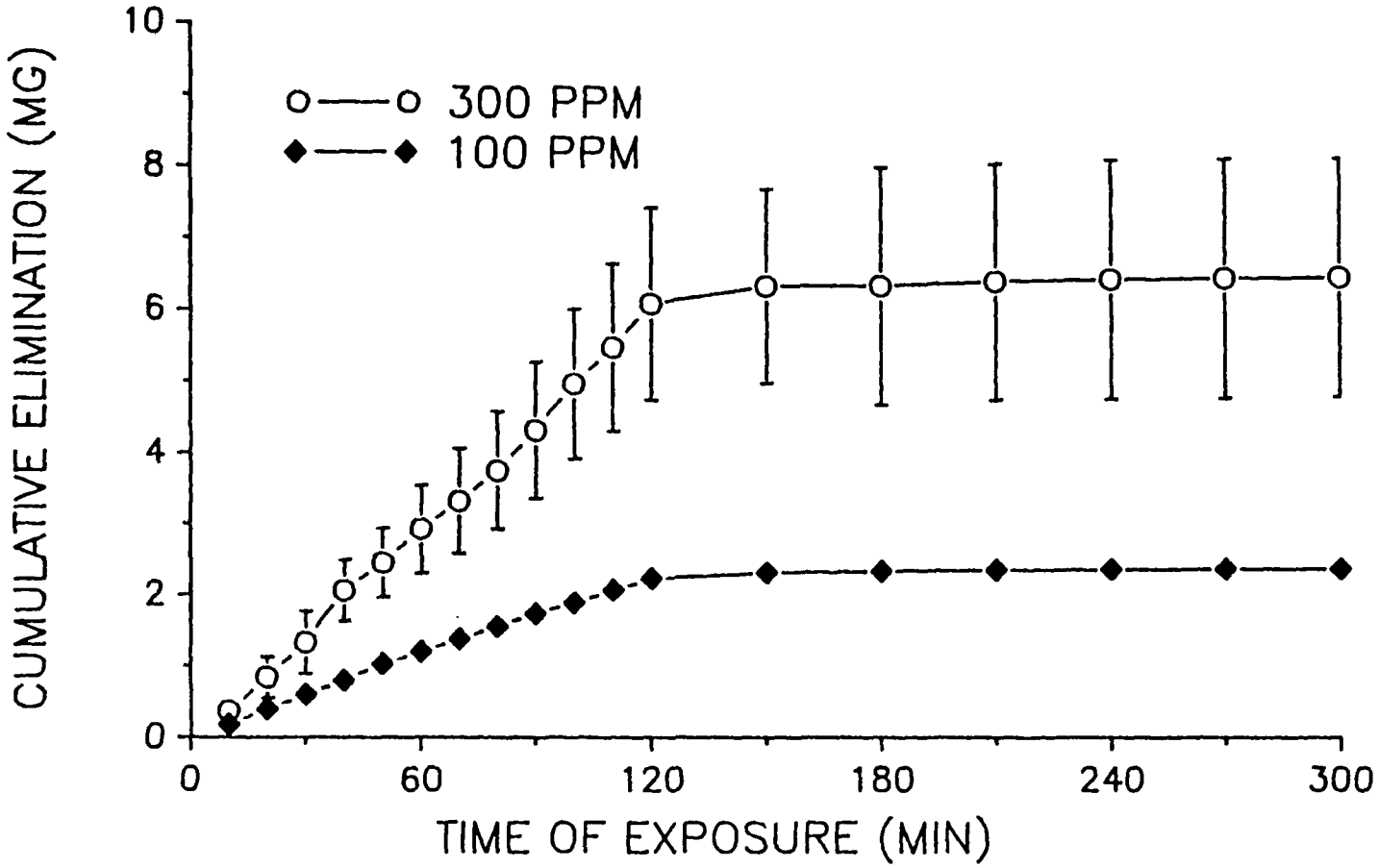
Cumulative uptake of DCE during inhalation exposures. Rats inhaled 100 or 300 ppm DCE for 2 hr. The quantity of inhaled DEC retained during successive 10-min intervals was calculated on the basis of the measured minute volume and difference between inhaled and exhaled TCE concentrations. Each point represents the mean ± SE for 6 rats.

Figure 4



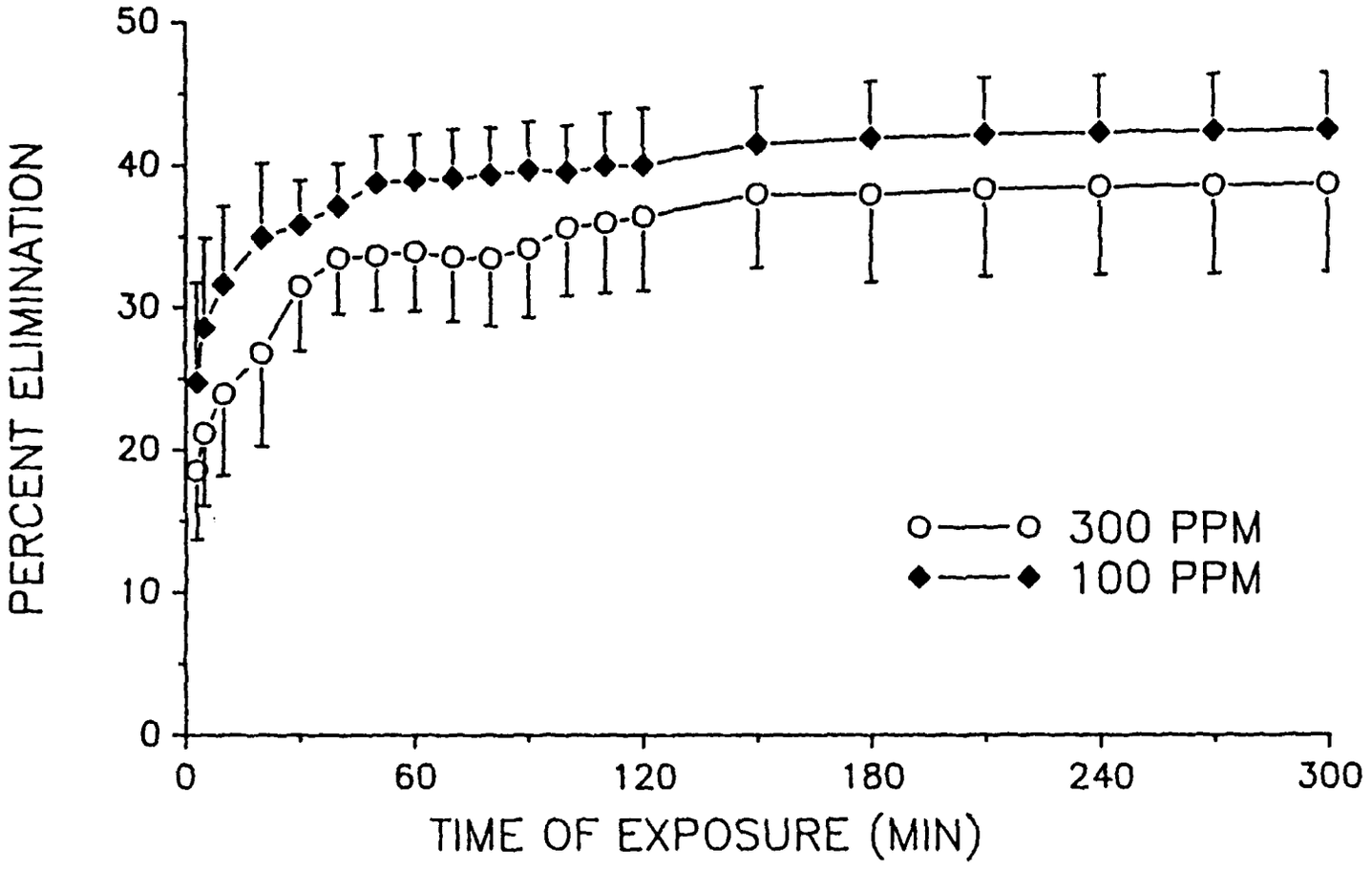
Percent uptake of DCE during inhalation exposures. Rats inhaled 100 or 300 ppm DCE for 2 hr. Each point represents the mean ± SE for 6 rats. The percent uptake of the inhaled dose over time was determined after 1, 3, 5, 10, and 20 min and at 10-min intervals thereafter.

Figure 5



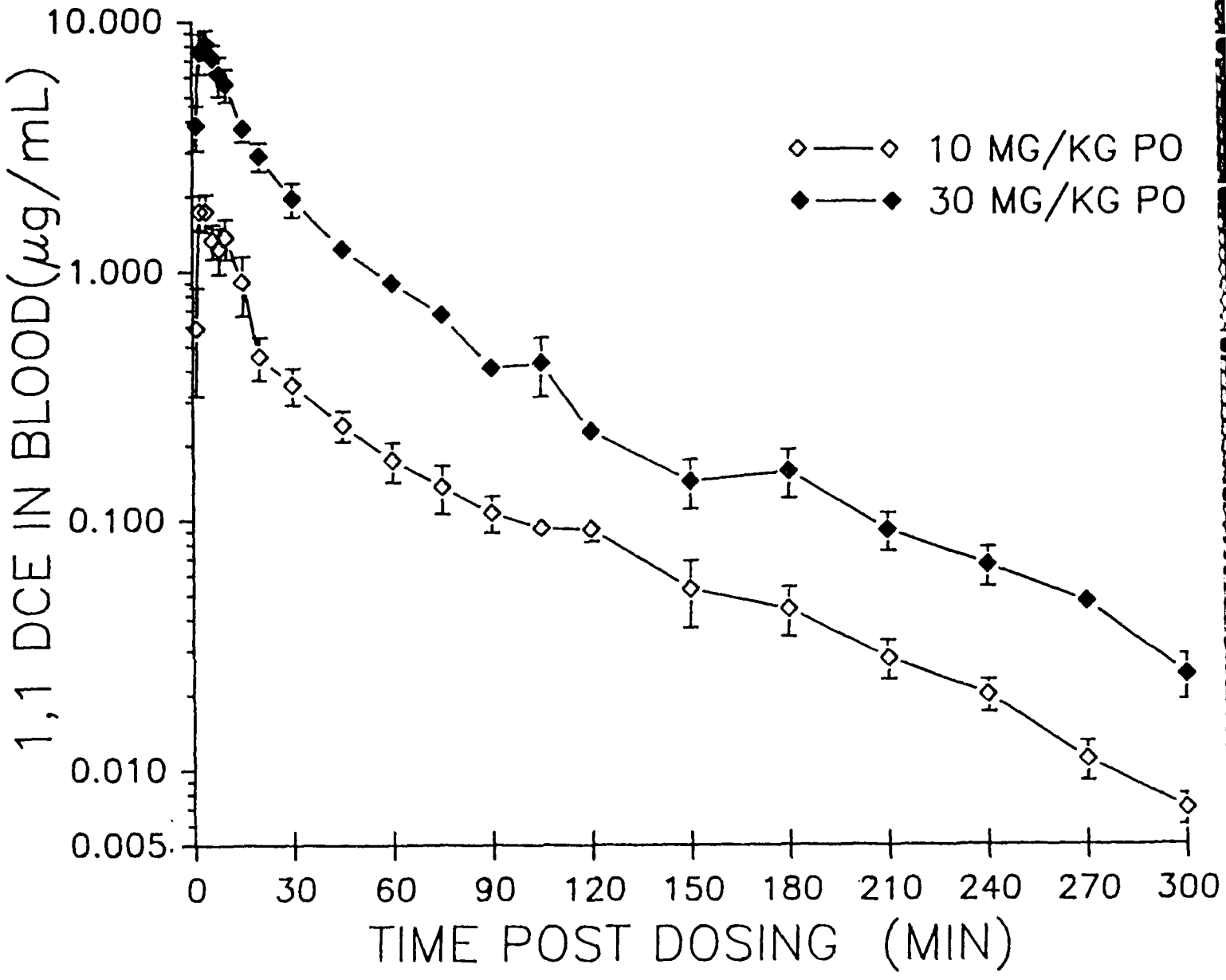
Cumulative elimination of DCE during and following inhalation exposures. Rats inhaled 100 or 300 ppm DCE for 2 hr. The quantity of inhaled DCE eliminated in the breath over time was calculated using direct measurements of the minute volume and DCE concentrations in the inhaled and exhaled breath. The contribution of inhaled DCE from instrumental and anatomic dead space to the quantity exhaled was deleted. Each point is the mean ± SE for 6 rats. Cumulative elimination was determined for successive 10-min intervals during the 2-hr exposure, and for successive 15-min intervals post-exposure.

Figure 6



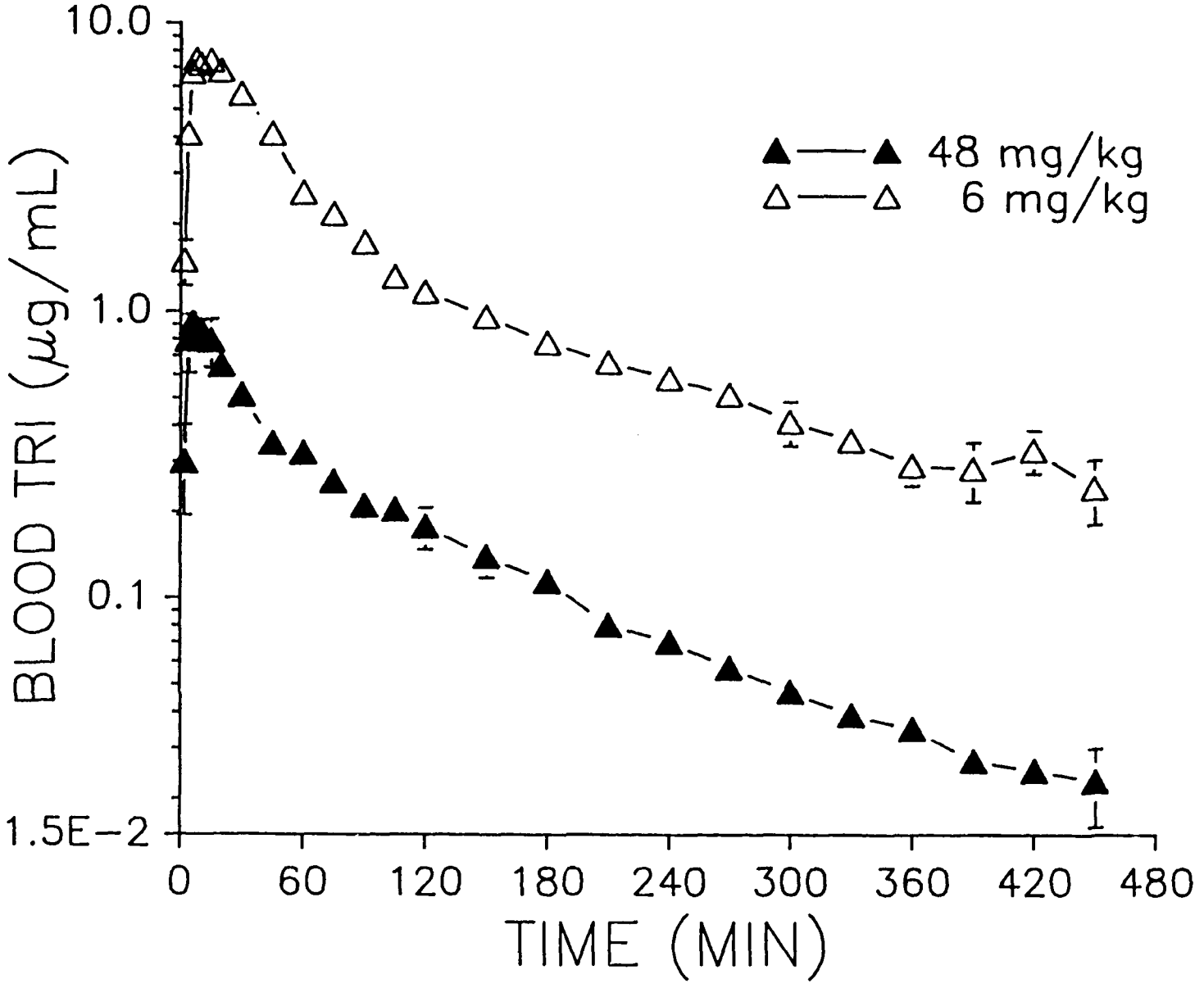
Percent elimination of DCE during and following inhalation exposures. Rats inhaled 100 or 300 ppm DCE for 2 hr. Each point represents the mean ± SE for 6 rats. The percent of the inhaled dose that was eliminated over time was determined after 3, 5, and 10 min and at 10-min intervals thereafter during exposure, and at 30-min intervals post-exposure.

Figure 7



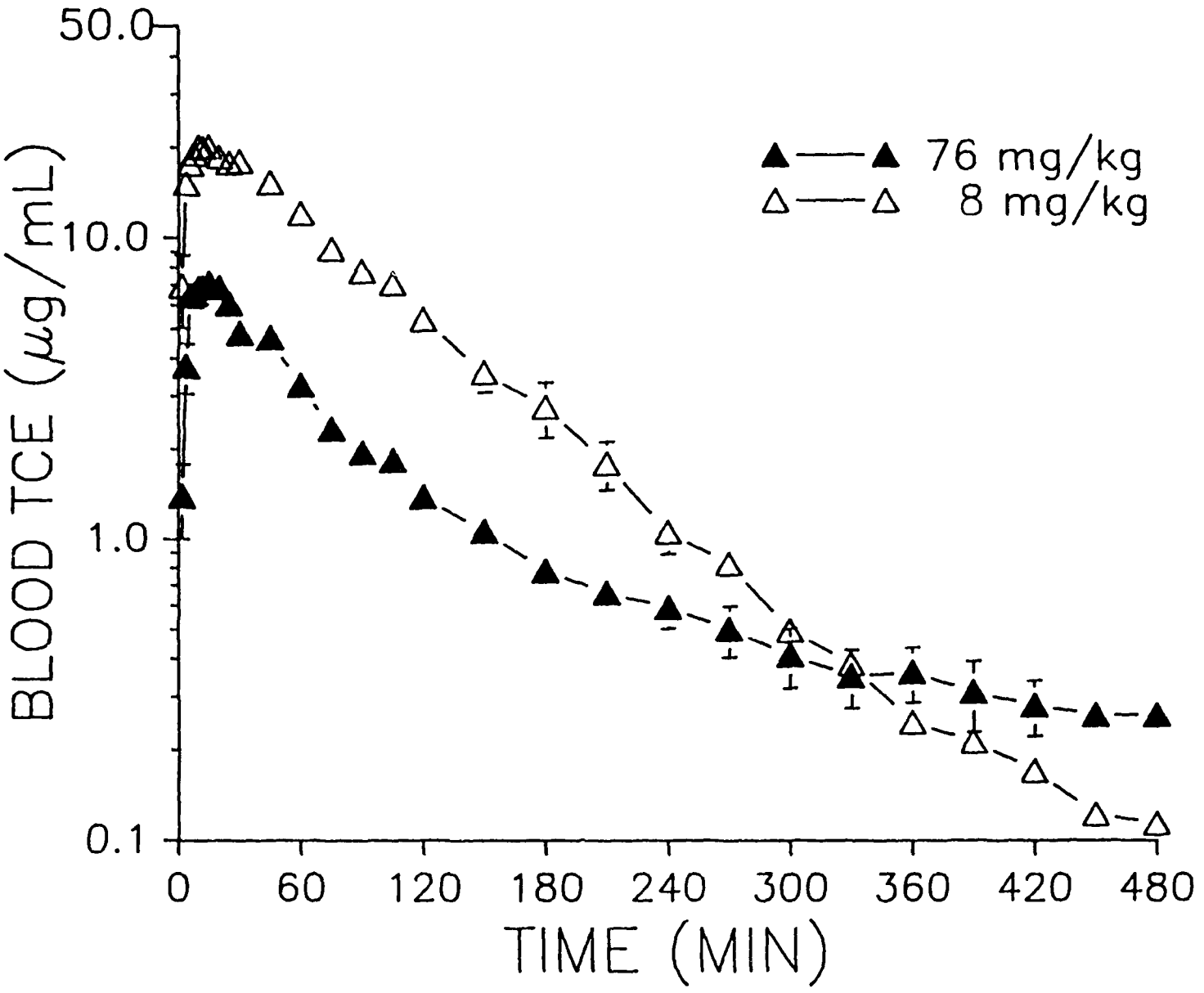
1,1-Dichloroethylene (DCE) concentrations in the blood of unanesthetized rats that have received a single oral bolus administration of 10 mg/kg or 30 mg/kg DCE in emulphor. Blood samples were taken from an indwelling carotid arterial cannula at 2 to 5 minute intervals during the rapid uptake phase and at 15 to 60 minute intervals during the elimination phase. Each value represents the mean ± SE for 6-8 rats.

Figure 8



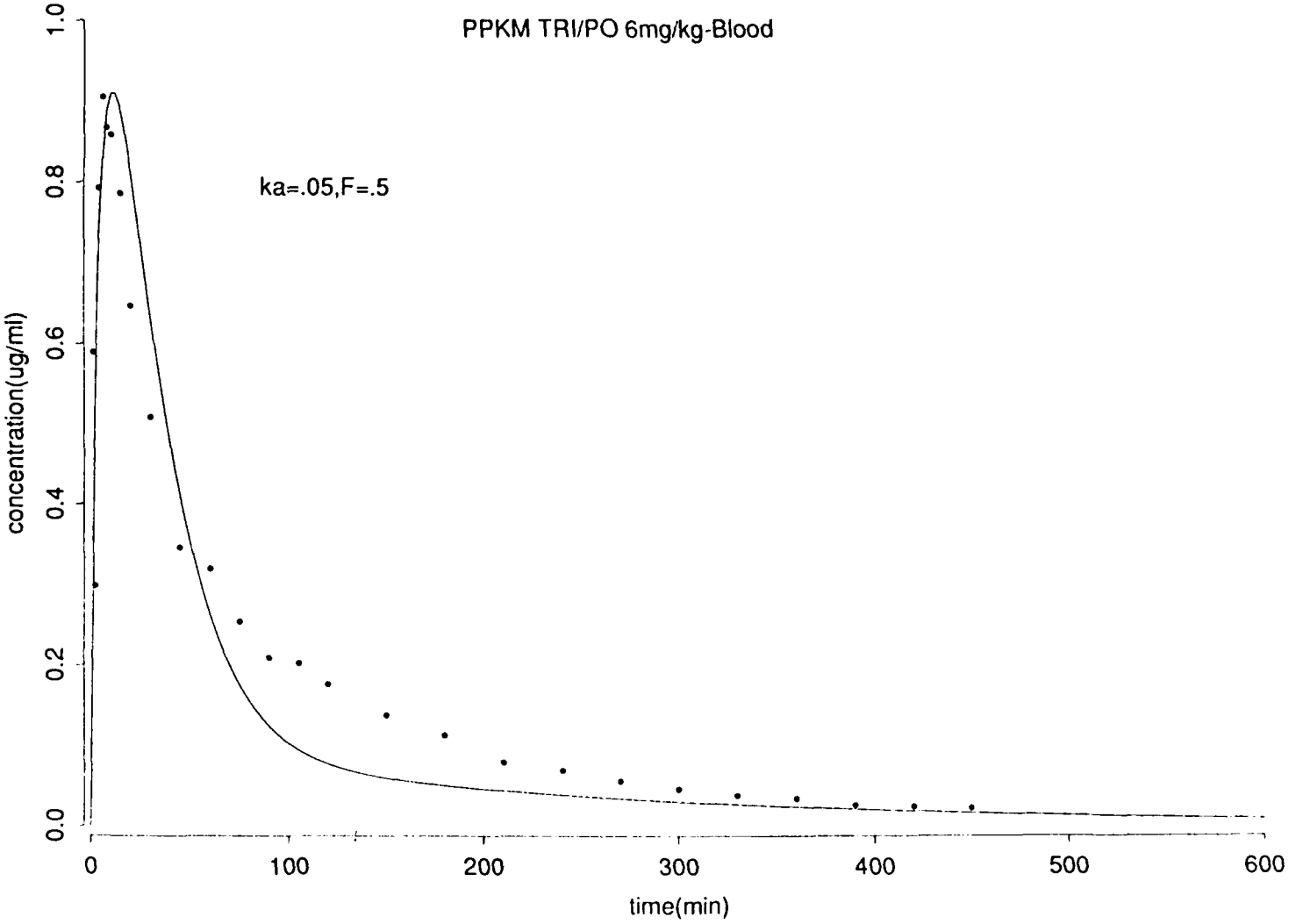
1,1-Trichloroethane (TRI) concentrations in the blood of unanesthetized rats that have received a single oral bolus administration of 6 mg/kg or 48 mg/kg TRI in emulphor. Blood samples were taken from an indwelling carotid arterial cannula at 2 to 5 minute intervals during the rapid uptake phase and at 15 to 60 minute intervals thereafter. Each value represents the mean ± SE for 6-8 rats.

Figure 9



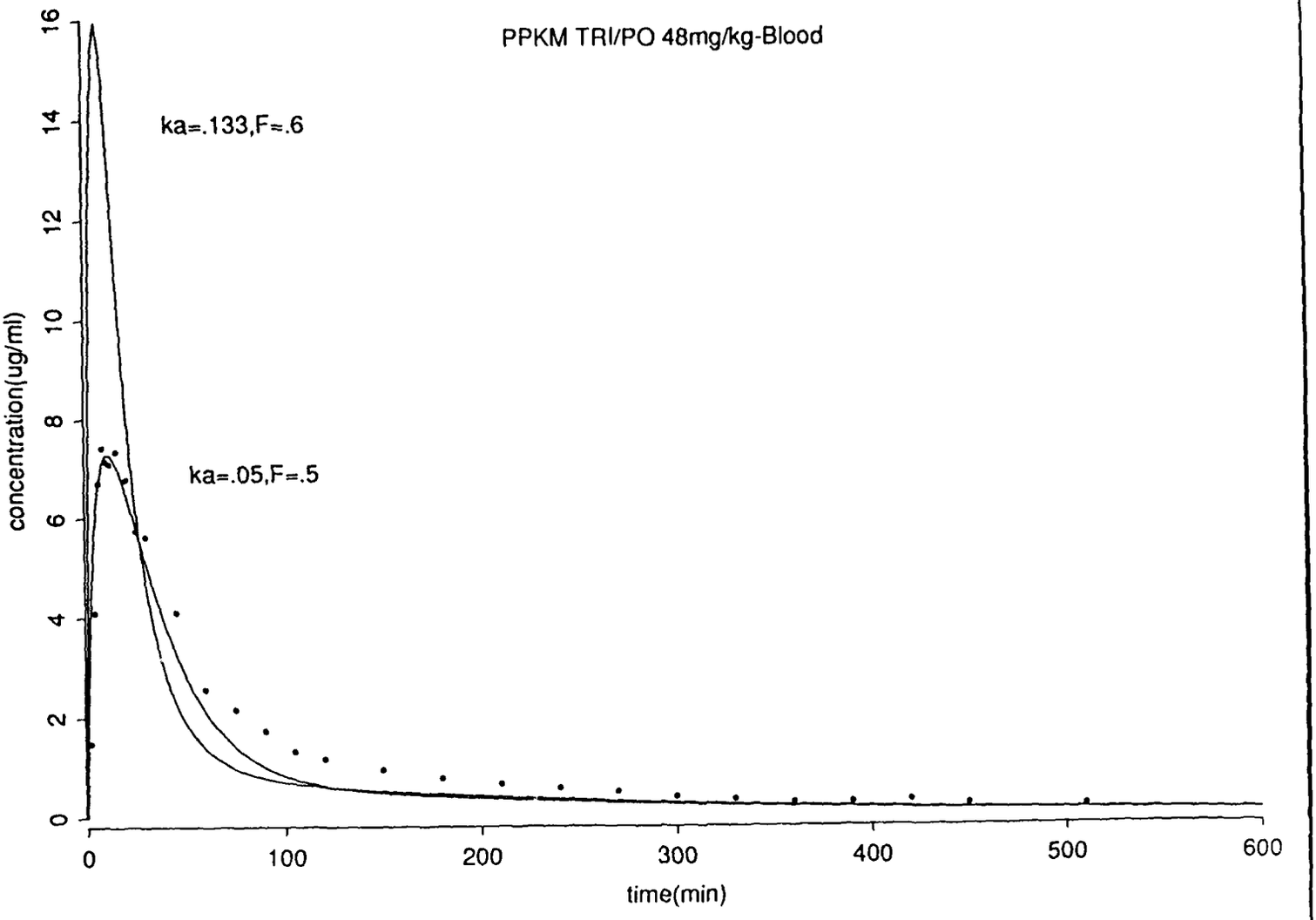
1,1,2-Trichloroethylene (TCE) concentrations in the blood of unanesthetized rats that have received a single oral bolus administration of 8 mg/kg or 76 mg/kg TCE in emulphor. Blood samples were taken from an indwelling carotid arterial cannula at 2 to 5 minute intervals during the rapid uptake phase and at 15 to 60 minute intervals thereafter. Each value represents the mean ± SE for 6-8 rats.

Figure 10



Observed (•) and model-predicted (-) TRI concentrations in the blood of rats that have received a single oral bolus administration of 6 mg/kg TRI in emulphor. In the physiologically-based pharmacokinetic model used for the simulation of TRI ingestion, a bioavailability of 0.5 and a Ka of 0.05 were employed. Each observed value is the mean for 8 rats.

Figure 11



Observed (•) and model-predicted (-) TRI concentrations in the blood of rats that have received a single oral bolus administration of 48 mg/kg TRI in emulphor. In the physiologically-based pharmacokinetic model used for the simulation of TRI ingestion, a bioavailability of 0.5 and a Ka of 0.05 were employed. Each observed value is the mean for 6 rats.

Figure 12

TABLE I

PHARMACOKINETIC PARAMETERS FOR 1,1-DICHLOROETHYLENE (DCE) FOLLOWING SINGLE ORAL BOLUS ADMINISTRATION IN UNANESTHETIZED RATS

	DOSE	
	30 mg/kg	10 mg/kg
@C-MAX (UG/ML)	9.6 ± 1.3	2.25 ± 0.28
AUC (UG.MIN/ML)	239 ± 23.7	50.5. ± 5.9
ELI.HALF-LIFE (MIN)	55 ± 4.6	50 ± 3.6
APP.CLEARANCE (ML/MIN/KG)	131 ± 12	216 ± 23
APP.VOL.DIST (L/KG)	10.67 ± 1.9	16.1 ± 2.5

Values are the MEAN ± SE for 6 to 8 rats.

@ DCE in arterial blood.

TABLE II

PHARMACOKINETIC PARAMETERS FOR 1,1,1-TRICHLOROETHANE (TRI) FOLLOWING SINGLE ORAL BOLUS ADMINISTRATION IN UNANESTHETIZED RATS

	DOSE	
	48 mg/kg	6 mg/kg
@C-MAX (UG/ML)	7 ± 0.5	0.8 ± 0.07
AUC (UG.MIN/ML)	646 ± 35	64 ± 6
ELI.HALF-LIFE (MIN)	115 ± 7	112 ± 4
APP.CLEARANCE (ML/MIN/KG)	76 ± 3.7	91.7 ± 8.0
APP.VOL.DIST (L/KG)	12.1 ± 1.7	19 ± 0.9

Values are the MEAN ± SE for 6 to 8 rats.

@ Concentration of TRI in arterial blood.

TABLE III

PHARMACOKINETIC PARAMETERS FOR TRICHLOROETHYLENE (TCE)
FOLLOWING SINGLE ORAL BOLUS ADMINISTRATION IN UNANESTHETIZED RATS

	DOSE	
	76 mg/kg	8 mg/kg
@C-MAX (UG/ML)	7.7 ± 0.8	1.50 ± 0.2
AUC (UG.MIN/ML)	936 ± 72	42.5 ± 2.8
ELI.HALF-LIFE (MIN)	116 ± 13	78 ± 1.6
APP.CLEARANCE (ML/MIN/KG)	85.6 ± 8.6	181 ± 16
APP.VOL.DIST (L/KG)	11.1 ± 1.0	21.7 ± 4.3

Values are the MEAN ± SE for 6 to 8 rats.

@ Concentration of TCE in arterial blood.

APPENDIX D

Cumulative List of Research Articles and Abstracts

on Research Completed in Year 1

Dallas, C. E., Ramanathan, R., Muralidhara, S., Gallo, J. M. and Bruckner, J. V. "The uptake and elimination of 1,1,1-trichloroethane (TRI) during and following inhalation exposures in rats. Submitted to Toxicology and Applied Pharmacology (1988).

Dallas, C. E., Ramanathan, R., Muralidhara, S., Gallo, J. M. and Bruckner, J. V. "Direct measurement of trichloroethylene (TCE) in the blood and exhaled breath of rats during and following inhalation exposures". To be submitted to Fundamental and Applied Toxicology (1988).

Dallas, C. E., Ramanathan, R., Muralidhara, S., Gallo, J. M. and Bruckner, J. V. "Comparative pharmacokinetics of inhaled and ingested 1,1-dichloroethylene (DCE) in rats". 27th Annual Meeting of the Society of Toxicology, Dallas, TX; Toxicologist 8: 139 (1988).

Muralidhara, S., Ramanathan, R., Gallo, J. M., Dallas, C. E., and Bruckner, J. V. "Pharmacokinetics of volatile halocarbons: Comparison of single oral bolus versus gastric infusion of 1,1,1-trichloroethane (TRI)" 27th Annual Meeting of the Society of Toxicology, Dallas, TX; Toxicologist 8: 95 (1988).

Ramanathan, R., Muralidhara, S., Gallo, J. M., Dallas, C. E., and Bruckner, J. V. "Pharmacokinetics of volatile halocarbons: Comparison of single oral bolus versus infusion of trichloroethylene (TCE)". 27th Annual Meeting of the Society of Toxicology, Dallas, TX; Toxicologist 8: 94 (1988).